1967

Mating and oviposition of Empoasca fabae (Harris) (Cicadellidae: Homoptera)

Oscar Verdell Carlson

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MATING AND OVIPOSITION OF EMPOASCA FABAE
(HARRIS) (CICADELLIDAE: HOMOPTERA)

by

Oscar Verdell Carlson

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

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Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1967
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INTRODUCTION

Mating and oviposition, which are primary phenomena in the continued existence of *Empoasca fabae* (Harris), were selected for intensive study. Laboratory conditions were devised to permit the observation and timing of events. Motion pictures were made to facilitate observation of behavior, and recordings were made of sounds produced by the leafhopper. Mating frequency and age limitations to mating were explored.

At selected times during oviposition, the processes were stopped abruptly and histological sections were prepared to aid in interpreting behavioral events.

The descriptive studies reported in this dissertation mark an initial step toward experimental analysis of leafhopper behavior and toward exploration of the biological significance of behavior in this species.
PART I. MATING

If sexual reproduction is to take place

... usually two conspecifics of different sexes must find each other and cooperate with one another. Primarily, the species and sex of the partner has to be properly chosen. Then a whole system of courtship behavior usually serves to stimulate the partners and to synchronize and direct their actions. Escape or attack responses must be inhibited to the point where the sexual consummatory act can be achieved. (Markl, H. and M. Lindauer 1965)

One component in the phenomenon of sexual reproduction, mate finding, is limited in this study by the close proximity of males and females within small cages and also by the inclusion of males or females selected from groups of known age or previous experience.

Procedures were devised to facilitate observing and recording pre-mating behavior (sound production, physical alignment, rejection of males by females, rejections of males by males), copulation (behavior, age limitations, and time in copulo), and post-copulatory actions of males and females.
MATERIALS AND METHODS

Leafhoppers for mating studies were obtained from a greenhouse culture maintained on broadbean, *Vicia faba* L., grown in screened cages, 24 X 24 X 28 inch, in the Iowa State University Insectary greenhouse. Reference specimens were preserved on points and in alcohol and were placed in the Iowa Insect Collection.

Usually nymphs were removed from greenhouse cages by use of an aspirator and transferred to plastic snap-box cages, 3.5 X 3.5 X 3.5 cm, (Fig. 1). Each cage had a 0.8 cm opening in the bottom to admit a segment of bean stem and a 0.6 cm opening (plugged with cotton) in one side for introducing and removing leafhoppers. Food was provided by a broadbean stem which protruded approximately 1 inch into the cage. The opposite end of the stem was immersed in a 3% sucrose solution contained in a floral water-pick. The bean stem was protected with cotton wrapping at points of contact with the water-pick stopper or the plastic cage. The stems were replaced with freshly-cut stems every 24 hr. Leafhopper adults were held for 4 months by this procedure.

If adult leafhoppers were required, nymphs were collected and up to 6 were confined in each cage. The cages were held in a controlled-environment cabinet at a daytime temperature of 26°C (r.h. 30%) and a night-time temperature of 18°C (r.h. 34%).

During motion filming and observing mating behavior, a shallow clear-plastic box, 4.8 X 4.8 X 0.6 cm, was used to confine the leafhoppers. The top, of single-strength glass, was attached to the base by transparent tape (Fig. 2). Holes (approximately 1 mm in diameter) drilled through the sides
and bottom provided ventilation or admitted thread to hold the leaf against the bottom. Of two openings (0.6 cm) in the side of the cage, one admitted a leaf petiole and the second provided for introducing leafhoppers.

A clear-plastic observation-cage, 2.9 X 2.9 X 1 cm, was used while monitoring leafhopper sounds. The bottom was replaced with a fine nylon cloth. A piece of bean leaflet was sewn to the cloth. The leaflet provided food satisfactorily for only 3-4 hr. The cloth surface of the cage was placed in contact with the microphone head. Usually 10 pairs of leafhoppers were placed in the cage.

Sounds were recorded on a Magnicord Model-14 tape recorder operating at 7½ inch per second. Background sound below 100 cyc/sec and above 15,000 cyc/sec was removed by passing the recorded sound through a Krohn-Hite band-pass filter, Model 310-AB. A Grass oscilloscope-recording camera Model-C-14 was used to photograph the sound-tracing from the screen of a Hewlett-Packard Oscilloscope, Model-1308.

Two different types of boxes for minimizing extraneous sound were used (Fig. 4). Sound recordings were made in the larger box, designed by Dr. K. C. Shaw to accommodate insects during sound recordings. Its construction included the following: beginning with the internal silent chamber (11 X 12 X 10-inch), which was surrounded by a 1½-inch layer of plastic-foam cones directed inward, there was a ½-inch layer of felt, a ½-inch layer of plywood, a 2-inch layer of exploded mica and plaster of Paris. These layers were encased in a box made of ½-inch plywood. Four metal rods suspended this inner box and there was a 6-inch air space between it and the outer box. The outer box was double-walled (formed of ½-inch plywood)
and filled between the walls with a 3-inch layer of sand. During record-
ings the box was closed with a unitized lid of the sound-proofing layers.

An American electro-dynamic microphone, Model-D33, was held firmly
inside the inner box. The mating cage (Fig. 3) was taped directly onto the
head of the microphone. Low intensity light was provided by a flashlight
suspended in the chamber with the insect cage. The temperature within the
sound box was 24 ± 1°C.

For simultaneously monitoring sound and observing the leafhoppers,
a modification of the box designed by Moore (1961) was built. Its design
and equipment are described, beginning with the interior: an American
electro-dynamic microphone (Model-D33) was surrounded with foam-rubber
packing 4 X 4 X 12 inch; outside of this was a 3-inch layer of Celotex®
(Celotex Corporation), 2-inch of glass wool, a 3-inch layer of plywood,
and a 3-inch layer of Celotex. The outside dimensions of the box were 8 X
8 X 14 inch. The lid was made of 3-inch plywood with a 2 3/4 X 2 3/4-inch window
in the center. The window (two layers of single-strength glass) was set
with epoxy cement within the 3-inch plywood. This part of the top fit
within the Celotex sides. On top of the plywood was glued a piece of
Celotex (8 X 8 X 3-inch) with a 2 3/4 X 2 3/4-inch center aperature aligned with
the window.

A dissecting microscope mounted on a universal arm was used to observe
the mating behavior while sound was being monitored (Fig. 4). A magnifica-
tion of 10X was usually satisfactory, although 7X and 25X were used also.

Mating behavior was recorded on 16 mm motion film. An American-
Optical cycloptic stereoscopic-microscope, Series 58, with 7X, 10X, and 15X
oculurs, was used with a single photographic-tube adapter, Model-638, to
pass an image of the insect to an Arriflex-16 camera fitted with a Periscope-Finder attachment. Intensity of light received at the film was measured by using a Microsix-L exposure-meter. Kodak plus-X negative film was exposed at the rate of 24 frames per second.

Two American-Optical adjustable microscope-illuminators with iris diaphragms, 2-lens systems, and 100-watt, 120-volt bayonet-base lamps, were used in conjunction with a Cyclospot, GE-1493, 6.5-watt bulb, to illuminate the leafhoppers for photographing. Heat-absorbing filters, placed in front of each lamp, were necessary. The photography-room temperature was 25 ± 2°C.
RESULTS AND DISCUSSION

Premating Behavior

Sounds emitted

**Sound-I**  As a male walked toward a female from a close range (approximately 2 mm), a sound (sound-I), which continued for about 3 seconds, emitted from the male. During a 3-second period there were typically 4 phrases of pulse repetitions (Fig. 5a).

Each phrase required from 240-280 msec and the phrases were separated by intervals varying from 520-700 msec. Seven to nine pulses make up each phrase. Each pulse required 10 msec and there was an interval of 20 msec between pulses. The first sound component within a pulse was relatively less in amplitude (Fig. 5a).

The paired sound-components of a pulse emitted by *E. fabae* show physical characteristics similar to those Pringle (1954) attributed to emissions of tymbal origin. Pringle (1954) reported that the two tymbal-components of cicada sounds were produced by the "in click" and the "out click" of the tymbal and that the "in click" produced a sound of smaller amplitude than did the "out click".

In my recordings of sound pulses produced by males of *E. fabae*, the amplitude of vibrations characterizing the initial component gradually increased from the base line up to the point of greatest amplitude, thereafter sharply diminishing to zero (Fig. 5b). The second component of the pair began suddenly at an amplitude exceeding the greatest amplitude of component one, without preliminary vibrations. This clean-cut initiation was followed by diminishing vibrations for about 2 msec. There were some
individual variations; in one recording the amplitudes of the paired emissions were equal (Fig. 5b), except that the first-recorded pair was unequal.

Sound-I frequencies of *E. fabae* ranged between 500 and 1500 cyc/sec. This was much lower pitched than that of the cicadas (Pringle 1954), but slightly higher than that of the leafhopper, *E. casta* (Moore 1961).


The vibration rate, 30-33/sec recorded from *E. fabae* was near the range of that from *E. casta*, 40/sec. at 28.5°C., reported by Moore (1961). Tymbal vibrations of cicadas varied from 120 to 600 per sec (Pringle 1954). Ossiannilsson (1949) considered that in the leafhopper, *Doratura stylata*, the pitch of the male call elevated with increased temperature.

George (1933) reported that there were internal apodemes, which extended from the second to the fourth abdominal segment, with attached muscles. He believed that sound was produced by flexure of the apodemes when the muscles contracted.

During the course of simultaneously observing leafhopper pairs and monitoring sound, I frequently observed that after the male emitted from 1 to 6 phrases it proceeded to copulate with the female. If the male was silent no copulation occurred, but sometimes, even after the male produced sound-I, the female rejected the male and no copulation ensued.
The sound was not produced by females caged together, but it was produced by males caged together.

In some cases copulation followed promptly after a single sound phrase was emitted and in other cases, only after three, four or six phrases were produced. This suggested that readiness to mate was influenced by the number of sound phrases from the male.

Is it possible that males were sexually excited by male sounds and stimulated to the action evident during the period of alignment?

- **Sound-II** A second sound (sound-II) was produced by males of *E. fabae*. Typically, this sound emitted from males quiescent following flight or ambulation. It was emitted with the abdomen raised and with a visible, rapid, accordion-like telescoping vibration of the 7th and 8th abdominal segments. The sound frequency ranged between 500 and 1500 cyc/sec.

When another leafhopper, often a male, came within a few millimeters, the volume and intensity of sound-II was notably increased. It was not resolved whether or not this reaction indicated a territorial warning, or if the sound served as a signal to females or other males.

The structure that produced sound-I (or sound-II) was not identified. However, the similarities in frequencies suggested that both sounds may be made by the same structure. By using different muscles to manipulate the one structure, it is possible that bending a tymbal may produce sound-II, whereas the sudden release and subsequent inflexing may produce the paired emissions of sound-I.

To my knowledge, these are the first reported records of sounds produced by *E. fabae*. 
Ossinnilsson (1946) first reported sound production in the genus *Empoasca*, and later described "a laughing sound" (1949) that was produced by *Empoasca viridula* (Fall.), caged in a glass cylinder inserted in his ear. Ross (1959) postulated that sound could be produced by muscular manipulation of internal apodemes of the first abdominal segment in *E. fabae*.

Physical Alignment

Premating behavior of the *E. fabae* males usually began with cessation of feeding, followed by rapidly walking sideways or forward until a female was encountered. As the male came within a few millimeters, his approach was slow and appeared cautious. He approached the female from the right, or left-posterior oblique, advancing until his head and the female's metathorax were in juxtaposition.

The wings of the male were brought from the normal inverted V (at-rest position) into a horizontal plane, and rapidly vibrated in this plane. This action lasted for approximately 3 seconds, a time corresponding roughly with that of sound production by the male. At this time, the sub-genital plates of the male were spread and the lateral parameres were in position for introduction into the ramal sac of the female.

The male's metathoracic leg closest to the female was placed over the female's wings as the posterior tip of the male's abdomen was flexed laterally toward and beneath the female's abdomen. The lateral parameres were extended and positioned so that the genital plate of the female was opened and the parameres thrust into the ramal sac of the female.
If the female was receptive she raised the posterior region of the abdomen, allowing the insertion of the male's clamping structures. As the male began to make a 180° turn, ultimately achieving a tail-to-tail position with the female, he released the metathoracic leg-hold and inserted the penis. The female would walk forward as the male released his leg-hold and made the turn.

After reaching the tail-to-tail position, the female adjusted her wings to a position dorsal to those of the male. A pumping action of the male's abdomen began and continued throughout copulation.

In further discussing physical alignment, it seems pertinent to consider the roles of sound, body movements, or other evidences of orienting stimuli. Fluttering or lateral movement of the male's wings, as well as sound production, may be courtship displays necessary to bringing the female into a breeding condition (Burton 1953). It is possible that the wing displays of *E. fabae* may have been important in inciting the female into copulation. The female's response to sound may be immediate, or follow only after prolonged stimulation, depending upon her intrinsic orientation toward mating.

While the male and female were feeding, prior to any evidence of male-female orientation, there was an intermittent dorsal-ventral vibration of the abdomen. This movement did not coincide with sound-II produced by the male. As far as I have been able to determine, the female is silent, although it is possible that tapping of the substrate with the ventral posterior region of the abdomen may provide some stimulus to other leafhoppers. McMillian (1963) noted that, following the abdominal vibrations by males and females of *Sogata orizicolor*, the male would begin to search
for the female. The role of such actions in the male-female orientation of
E. fabae will require further study.

While the male was making the 180° turn the female often began to walk
forward. Whether this was an escape act or a cooperative act, it served to
help the male complete the 180° turn.

Disruptions of the Mating Pattern

Rejection of males by females

There were several typical actions of the leafhoppers that disrupted
the mating pattern, with the result that copulation was not accomplished.
Of 70 attempts that I observed during one period of 52 minutes (9:07-
9:59 pm, 23 September, 1966, in greenhouse rearing-cages at 25°C) only one
attempt ended in copulation.

The following are descriptive of typical disruptions to mating:
1. When the male approached the female, he followed a pattern of
   wing flutter and sound production, but upon moving into closer alignment,
   the female physically snapped the male away by a sudden sharp strike with
   her metathoracic leg. This resulted in either one or both participants
   being dislodged from the substrate.

2. The male approached the female, followed through a pattern of wing
   flutter and sound production, and then laterally flexed the posterior tip
   of the abdomen into position to insert the lateral parameres. As he did
   so he placed the metathoracic leg over the female and then began to make
   the 180° turn. At this point, the female walked forward rapidly, before
   the parameres were inserted and in spite of the leg hold. This action often
dislodged the male.
3. The male occasionally approached a copulating pair, followed through the pattern previously described, ultimately spreading his sub-genital plates and attempting to insert the parameres. The male was persistent but copulation was impossible because the female was in copulo with another male. The copulating pair usually moved away from the intruding male, but were not as motile as the single individual. The intruding male would finally release his metathoracic leg-hold, and often he would repeat the pattern with the same pair.

Rejection of males by males

Under conditions of the small cages, and in the large greenhouse rearing-cages as well, male leafhoppers apparently attempted to copulate with other male leafhoppers. The male behavior-pattern, previously described in relation to the female, was accomplished with a male. The rejecting male reacted like the rejecting females in examples 1 and 2 described previously.

Male to male contacts in leafhoppers offers a phenomenon that could be exploited in exploring the roles of suspected stimuli in mate finding. Desmond (1952) has described the courting of a male stickleback fish by another male as homosexuality. In that case the scarcity of females in the aquarium caused aggressive males to accept other males as mating partners. Miller (1950) found that male Drosophila courted males of the same species about as frequently as they courted females. Although crowding of leafhoppers or a scarcity of females may add to the possibility of males contacting males as prospective mating partners, the occurrence of frequent male-to-male contacts suggested that stimuli, strongly reinforcing to mate-finding, may be lacking in E. fabae.
Copulation

The abdomens of leafhopper pairs in copulo gently undulated continuously in the horizontal plane. Frequently, the female lifted and spread apart the wing tips of the male with her metathoracic legs. During this action the tibia of the metathoracic legs brushed along her wings, while the tarsi were moved posteriorly-ventrally, touching the setae of the male's genital plates. This action was more frequent near the end of the time in copulo.

The physical interlocking of the male's lateral parameres and aedeagus with the female's genital plate formed a strong holding-apparatus (Fig. 9). Once established, the connection was not usually uncoupled during ambulation, flight, disturbance by extraneous males, nor during capture with an aspirator (a fairly jostling treatment).

Uncoupling the apparatus at the termination of copulation must require a certain amount of cooperation.

Histological sections through the genital apparatus of copulating pairs, suddenly killed with hot (65°C) paraffin (M.P. 60-63°C) and embedded by Davenport's (1960) double-infiltration method, indicated the following:

1. During copulation the genital plates of the female formed a wedge between the parameres and the penis, structuring a very strong interlocking mechanism. Kershaw (1910) reported that the sacs on either side of the penis were dilated by blood pressure and that they served to hold the female securely during copulation. Cunningham's (1962) illustration of the interpositioned aedeagus and lateral parameres of *Empoasca maligna* was
helpful in clarifying my understanding of the interlocking mechanisms of 
*E. fabae*, for they are similar.

2. Only the distal end of the aedeagus appeared to enter the female's 
genital tract (Fig. 8). There was no evidence that the aedeagus extended 
to or entered the spermatheca.

3. A fluid (eosin-positive) was apparent in the female genital tract 
and subsequently entered the spermatheca. In some cases sperm cells pre­
ceded it into the spermatheca (Fig. 9), and in some cases it was present 
without sperm cells.

Kershaw (1910) and Myers (1928) described the swollen walls of the 
ejaculatory ducts of some Homoptera (Auchenorrhynca) and suggested that the 
enlarged cells produced ejaculatory fluids which were moved into the 
ejaculatory bulb by slight pumping action.

**Age limitations to copulation and frequency of copulation**

**Males: Experiment I** In tests to determine how soon after adult 
emergence a male could mate, 30 male leafhoppers 1-day old were used. The 
males were placed in separate cages with 2 virgin females, 5-days old; 
there were no matings. On each successive day the males were transferred 
to fresh cages containing 2 virgin females, each 5-days old. On the second 
day, 2 of the 30 males mated; 17 of the remaining 28 males mated on the 
third day. Of the remaining 11, 6 mated for the first time on the fourth 
day, leaving 5 males which were considered old enough to mate after 5 days. 
All the mated females deposited viable eggs.

Sclerotization of the external reproductive structures would be 
requisite to the successful grasping by the parameres and the insertion of
the penis into the female apparatus. If all of the chitinous structures were not fully formed and hardened, or lacked well-bonded muscle attachments, copulation would seem to be impossible. In addition, fertilization of eggs would depend upon the definitive development of the internal reproductive system.

Helms (1967) found spermatozoa in the seminal vesicles during the first day of adult life in the potato leafhopper. Therefore, except for the limitations just discussed, the leafhopper probably could transfer sperm during the first day of the adult stadium.

Experiment 2 Tests were conducted to determine how many times the males would mate, and how long they would remain in copulo with successive matings. In these tests, 7 week-old males which had mated in the forenoon were each introduced into individual cages containing 2 virgin females, to determine if the males would mate a second time in the same day. Of the 7, 6 mated during that afternoon. Embryos and nymphs developed from the eggs laid by the females.

Even though there were only about 5 hr separating the mating periods, the males still produced enough sperm to fertilize the eggs of a second female. It was not determined whether or not the second female received enough sperm to fertilize all of the eggs produced during her lifetime.

In experiments carried out by McMillian (1963) S. orizicola males mated with as many as 3 females in 8 hr.

Experiment 3 In another test, 35 males which had mated once were individually placed in a holding cage (Fig. 1) with 2 females, 4 to 5-days old. The females were replaced daily to increase the possibility of at least one female being receptive to mating during that day. Twenty-two
males mated the second time. The number of males was reduced, by death or escape, to 18 by the sixth mating. Of the 18, 11 survived to mate 8 times. One male survived long enough to mate with 15 females. Viable eggs were deposited by all females that copulated during this experiment.

Multiple matings by males would enhance the reproductive capacity of E. fabae since they would tend to insure fertility of many females encountered. Multiple-matings of males has been noted in Erythroneura (Varty, 1964, 1967).

**Experiment 4** In experiments conducted to determine at what age the males ceased mating, males were placed in holding cages on the day of emergence. On selected dates, individual males were each caged with 2 virgin females 5-days old. Of 15 males 1 month old, only 1 mated, (others attempted to mate but were resisted), at 2 months 2 of 12 males mated, and at 3 months 7 of 17 mated. Viable eggs were deposited by all of the females except two that died shortly after mating.

**Females: Experiment 1** To determine the youngest age at which the female will mate, 30 females, which were 1-day old, were introduced individually into cages. Two 5-day old males were introduced into each cage. The one-day-old females did not mate. On the second day, one male attempted to copulate with a female, 2-days old, but was resisted. On the third day, 10 of the 30 females mated and at 4 days, 12 of the 20 remaining mated.

It was concluded that females were mature enough to mate after two days following the final molt. Prior to the second day of the adult stadium, the ovipositor and the rest of the exoskeleton are green colored. During the second day chitinous structures harden, and by the third day
the ovipositor becomes tan or brownish. It is at this time that the female begins to oviposit.

Renner (1952) found that newly emerged grasshoppers, E. brachyptera, avoided copulation, becoming receptive only after the ovaries had passed eggs into the lateral oviducts. McMillian (1963) found that day-old female S. orizicola did not mate, but that females 5-days old mated with males 5-days old. Since E. fabae can oviposit after 3 days in the adult stadium, receptivity to mating may depend upon passage of the egg into the lateral oviduct.

**Experiment 2** In tests to determine if the females mated more than once, 35 female leafhoppers were used. The first mating occurred when they were 4- to-5 days old. When the females were 16- to-17 days old they were introduced into cages with males 4 or 5-days old. Two females mated a second time on the first day; one female mated a second time on the second day. During 11 days, 15 females mated a second time, but they did not mate a third time.

If the female tract provided a favorable environment for sperm survival, it is probable that one copulation provided enough sperm for all the eggs produced by the female. Is it possible that additional matings occurred only after some of the seminal fluid and sperm had been emptied from the spermatheca?

Raine (1960) reported that the female bramble-leafhopper mated a second time after an interval of several days. Another homopteran, S. orizicola, no longer exhibited the behavioral characteristics of virgins after mating (McMillian 1963).
Experiment 3

Tests were conducted to determine the age when the female was no longer receptive to mating. Females, 30, 60, 90 and 120 days old, mated with males, 4 to 5-days old. Of 67 matings only one did not produce viable eggs. The females that were 120 days old died about one week after this mating.

Time in copulo

In determining the amount of time spent in copulo, the leafhoppers were observed every 15 min or less.

In continuous observations there was no case in which the period of mating was less than 30 min. There were few matings that lasted as little as 30 to 40 min and few leafhoppers remained in copulo more than 95 min. The periods of time in copulo varied from 30 to 139 min, the average time of 155 matings was 84 min.

One male leafhopper that had mated 8 times previously remained in copulo for: 105, 75, 90, 65, 55, 95, and 55 min from the 9th to the 15th mating respectively. The sampling was too small for conclusive evidence, but it seemed likely that the amount of time spent in copulo did not increase with successive matings.

McMillian (1963) reported that S. orizicola males in their first mating completed copulation in 3 sec but that they required progressively longer for successive matings.

Postcopulation

Separation of the male and female after copulation usually included a forward movement of one or both of the individuals. In one case closely
observed, the male released the female after she had moved rapidly from side to side. In other cases, the female made no discernible movement just prior to separation.

The signal for release of the partner may be in the frequency of the female's touching the setae on the subgenital plate of the male, or in the short lateral movements of the female.

After the female was released, both male and female preened for a time. While the female retained a tripod stance, she lifted one, two, or three legs at a time and brushed various parts of the body. The antenna and the vertex of the head were frequently stroked with the antenna-cleaner on the tibia of the prothoracic leg. The mesothoracic leg was raised to stroke the metathoracic legs, and the ventral parts of the thorax and abdomen. The eye and surrounding area were stroked by both prothoracic and mesothoracic legs. The wings were stroked vigorously with a resultant straightening, unfolding and repositioning. The tibia of the metathoracic leg was brought dorsally and anteriorly to brush the dorsal surface of the body. The contact stroke began at the prothorax and extended posteriorly. With the same leg the under-surface of the abdomen also was briskly stroked.

The amount of time spent in preening varied considerably among individuals. I observed one male that preened vigorously for one minute, ceased briefly, then resumed preening vigorously for eight min.

Hill (1960) reported that females of Anthocoris sarothamni concluded copulation by moving into a crevice, thereby dislodging the male. This action was followed by the female "cleaning" her antennae with her front tarsi. Ossiannilsson (1953) observed that after the female Paropia scanica
separated from the male, other males attempted to copulate with her but were resisted. In my observations of *E. fabae*, copulation was not repeated immediately.
PART II. OVIPOSITION

Toward the ultimate objective of an analysis of oviposition behavior in *E. fabae*, a beginning is made in this study with a descriptive record of the phenomenon. Such a description brings to view events in the oviposition process that may be of interest for analytical study, and suggests those of special significance to the insect's ecology.

Laboratory situations were devised to permit observation and motion-picture recording of oviposition. The position of the egg during the process of transfer from the female body to a position within host-plant tissues was observed in histological sections of specimens killed during the act of ovipositing.

Limited experimentation with the leafhopper's oviposition responses to synthetic substrates illuminated something of the complexity of oviposition behavior, and suggested clues to mechanisms of its release or inhibition.
MATERIALS AND METHODS

Females for oviposition studies were collected from the greenhouse culture described on page 3. The insects were held individually in cages 3.5 X 3.5 X 3.5 cm (Fig. 1) for 3 days to allow them to oviposit. The bean-stem segment exposed to ovipositing females was cut free and cleared in hot lactophenol (Carlson and Hibbs 1962). Eggs were counted and leafhoppers which had deposited an average of 5 to 11 eggs/day were kept for experiments.

Leafhoppers were confined, during observation and motion-picture recordings of oviposition, between two microscope-slides positioned on either side of a bean-stem segment. A 3 mm Plexiglass® (Rohm and Haas Company) spacer with 0.39 mm holes formed one side and 2 small pieces of Plexiglass formed the ends (Fig. 6). Transparent adhesive-tape held the slides in place. The bean stem extended from the cage into a floral waterpick containing 3% sucrose. The cages were held at 24°C, a temperature that O'Keefe (1965) found optimal for oviposition. Light intensity was kept at the minimum necessary for viewing the leafhoppers through the dissecting microscope.

A second type of cage was used to permit observation and flooding the ovipositing female with hot Duboscq fluid. This flooding technique promptly interrupted the oviposition process at selected points. The cage was comprised of a clear-plastic base, 2.2 X 2.2 X 0.5 cm, with a glass microscope-slide covering. A hole was cut at one corner to receive a segment of bean stem for leafhopper feeding and oviposition. Another hole (plugged with cotton) was provided to admit the female and subsequently the
hot Duboscq fluid (Fig. 7). The bean stem extended beyond the cage into a floral water-pick containing 3% sucrose.

Modified Duboscq fluid (15 ml acetic acid, 150 ml ethyl alcohol, 60 ml formalin) (Galigher and Kozloff 1964), was heated and held for use at 70°C (Picric acid was omitted to prevent staining the microscopes). Galigher's FAA (op. cit.) was used in the same manner as Duboscq fluid.

At a selected time, the hot fluid was squirted into the cage, killing the female so quickly that the ovipositor was not removed from the plant tissue. If cold Duboscq fluid was used, the female withdrew the ovipositor from the plant.

After the female was killed, the stem segment with the adhering female was cut free and held in Duboscq fluid at room temperature for 24 hr. The specimen was transferred to 70% alcohol. Ten such specimens were dehydrated and embedded by Davenport's (1960) double-infiltration method.

Stairs' (1960) procedure, modified by substituting Paraplast® (Biological Research, Inc.) reduced the infiltration time and minimized breaking or shattering of the egg during sectioning. Histological sections (10μ) of the ovipositor, and of the ovipositor during passage of an egg, are illustrated in Figures 15 through 19.

The whole mount of the ovipositor was prepared by killing the female with Duboscq fluid, dehydration in an ethyl-alcohol series through 70%, severing the ovipositor from the body, and continuing dehydration through 100% ethyl-alcohol, and defatting in carbol-xylol. The ovipositor was placed in xylene and finally mounted without staining in Permoul® (Fisher Scientific Company).
Ovipositor cross-sections (10μ) were mounted on slides, stained regressively with alum-hematoxylin, and counterstained with fast green or eosin Y. These sections are illustrated in Figures 15-19.

Illustrations of body positions (Fig. 10, 12, 20 and 21), were prepared from the motion-picture record of oviposition (Carlson et al. 1967).

A synthetic medium acceptable for oviposition was sought. Three media were tested: 1. A 3% agar—3% sucrose medium was heated, cooled, cut into 3 x 3 x 12.5 mm blocks, and then covered with Parafilm® (Marathon Division, American Can Company). This synthetic "stem" was placed in an observation cage (Fig. 7). A piece of bean stem with its end abutting the Parafilm-covered agar "stem" could be covered by sliding aluminum foil over the bean stem, gently forcing the female (about to release the ovipositor) off the bean stem and onto synthetic agar "stem". 2. Vicia faba stems were macerated; the expressed fluid was mixed with 3% agar and 3% sucrose; the mixture was heated, cooled, and then cut into small blocks (as described above) and covered with Parafilm. This medium was placed in the cage as described above but without the addition of the fresh bean-stem. 3. Macerated bean-stem, plus 3% sucrose and 3% agar were heated, cooled, and then cut into small blocks (as above) and covered with Parafilm. The macerated bean-stem was the only food available to the female.

A stem of Solanum chacoense (considered relatively unacceptable for oviposition) was introduced into the observation cage (Fig. 7), in order to see if the behavioral pattern of oviposition was altered relative to the pattern on the acceptable broadbean.
RESULTS AND DISCUSSION

Preoviposition

The leafhoppers fed almost continually, occasionally interrupting feeding to walk, fly, rest briefly, mate, preen, or oviposit. Just prior to oviposition (often while the female had the feeding stylets inserted in plant tissues) movements of the ovipositor began. The action involved the first 4 parts of the ovipositor. The 2 lateral (first) valvulae and the 2 median (second) valvulae were moved anteriorly-posteriorly within the sheath formed of the third valvulae. Flexing of the ovipositor caused its mid-region to protrude from the sheath, from a longitudinal separation along the mid-region of the sheath, but the distal end remained secured within the sheath. This anterior-posterior movement and flexing of the ovipositor continued from 3 to 30 min prior to unsheathing of the ovipositor.

Females, abruptly killed and fixed just after the ovipositor was inserted into the plant tissues, had the egg in position to pass through the genital chamber (Fig. 11).

Within 30 min following the movements of the ovipositor just described, the female usually abruptly discontinued feeding, moved forward, raised the abdomen high and fully released the ovipositor from the sheath (Fig. 10). The ovipositor was brought forward forming approximately a 90° angle with the body. The mouth parts were usually touching the plant but were not inserted.

Other investigators have reported their observations of oviposition by homopterans. Snodgrass (1921) reported that the seventeen-year locust
required 25 min to excavate the egg chamber and to deposit eggs in a
tree twig. *Telamona compacta* (Membracidae) spent 2 hr and 40 min in pre-
oviposition activity before inserting the ovipositor into the stem (Dennis
1961). The preoviposition activity did not include an anterior-posterior
movement of the ovipositor. Readio (1922) observed a preoviposition action
wherein the ovipositor was held vertically just before it penetrated the
plant. According to Raine (1960) the ovipositor of the bramble leafhopper
was inserted and withdrawn several times before ovipositing. Varty (1967)
reported that the mouthparts of one leafhopper he observed were inserted
into the plant while the ovipositor cut a slit into the epidermis.

**Functioning of the Ovipositor**

The ovipositor began to penetrate the epidermal cells by means of a
sawing action, bringing into play the distal, rigid, toothed, dorsal-
surface of the second valvulae (Fig. 14, Cu.E.). Strong muscle-action was
evident in the third valvulae as the egg cell was cut into the tissues of
the plant. After the egg cell was cut, the female remained motionless with
the ovipositor in position (Fig. 12). Every few seconds the second and
third valvulae were moved slightly while the ovipositor remained deeply
inserted within the plant tissues.

The angle between the third valvulae and the substrate surface was
about 35° after the ovipositor was in the plant (Fig. 12). This angle
occasionally was increased if the female brought the ovipositor forward and
the tip failed to penetrate. In such cases the tip of the ovipositor slid
posteriorly entering the plant behind the initial point of contact. This
resulted in an angle of $40^\circ - 90^\circ$ between the third valvulae and the substrate.

In cutting the egg cell, the lateral valvulae functioned in supporting and strengthening the median valvulae. A tongue-and-groove mechanism (Fig. 15a, T.G.) extending from the anterior base of the ovipositor to within 200$\mu$ of the distal end, linked the median and lateral valvulae on either side, and allowed anterior-posterior movement of the second valvulae. The linkage of the first valvulae with the second valvulae formed a strong shaft effective in cutting and penetrating plant tissue.

A tongue-and-groove mechanism (Fig. 15a, T.G.L.V.) united the two halves of the ovipositor ventrally. This ventral interlocking mechanism was continuous posteriorly from the base of the ovipositor for about 510$\mu$. From 510$\mu$ to about 770$\mu$ the ventral connection was membranous and served to guide the egg into the egg cell in the plant. The membrane surrounded the egg but did not interlock nor fuse ventrally. The membrane gradually shortened ventrally until it terminated approximately 60$\mu$ from the distal end of the ovipositor.

On the external surface of the lateral valvulae there were ridges that came into contact with plant tissues when the ovipositor penetrated the plant. Following insertion of the ovipositor these ridges anchored the lateral valvulae during the plunging thrusts of the median valvulae.

The dorsal regions of the median valvulae were fused posteriorly (Fig. 16a, F.A.) for about 350$\mu$ from the proximal end. This fusion served to hold the two halves of the ovipositor together and also limited the dorsal expansion of the egg as it passed into the plant.
The sculptured area on the chitinous flap (Fig. 16b, T.A.) changed from a gently ridged portion anteriorly to a file-toothed structure in the posterior third of the ovipositor (Fig. 18b, T.A.). The toothed structures were recurrent almost to the end of the ovipositor. The egg, in passing through the ovipositor, was in contact with the ridges throughout its length (Fig. 18a, 18b; 19a, 19b; T.A.). After the leading end of the egg was in the ovipositor, the quick movement of the second valvulae and the file-toothed surface (acting as a ratchet) moved the egg posteriorly into the egg cell previously cut into the plant (Fig. 13).

As the tip of the ovipositor was withdrawn from the egg cell the egg was released from the ventral, posterior region of the ovipositor. The lateral valvulae moved very little while the egg cell was being cut.

Balduf (1933) reported that the flaps extending mesad from the median valves delimited the space which the egg occupied. He also theorized that the egg laid by *Draeculacephala* was released along the posterior edge of the ovipositor then was moved sideways into the puncture in the plant. The egg tube formed by many folds in the membranous portion of the lateral valvulae in the ovipositor of *E. fabae* is similar to the egg passage that Ekblom (1926) described in *Salda saltatoria*.

Readio (1922) and Brittain (1923) suggested that the tongue-and-groove mechanism allowed the median and lateral valves to slide independently but prevented their separation.

The great constriction of the egg (from 170µ to 65µ, Fig. 13) as it passed through the ovipositor was accomplished by deep convolutions of the chorion (Fig. 17b, Ch.E.) and chorion flexibility that allowed enlargement at either end of the egg.
On either side of the first valvular interlocking mechanism there was a flexible area (Fig. 16, 17, Fl.A.) which permitted a certain degree of expansion as the egg passed through. The membrane mesad to the alum-hematoxylin-positive area (Fig. 15a,b; 17a, St.A.) was pushed laterally, further accommodating the egg.

The ovipositor was so flexible, just posterior to the point of fusion of the second valvulae, that the passing egg bowed the two lateral halves of the ovipositor while the distal tips of the two halves remained in contact (Fig. 20). Then, as the egg continued into the distal ovipositor-tips, they separated leaving the egg in the plant.

Postoviposition

The egg was released during the final 30 to 50 sec of the oviposition period with quick anterior-posterior motions of the second and third valvulae.

After the egg entered the plant the convolutions of the chorion unfolded and the egg assumed its turgid, ovoid shape.

After the egg had been released the distal end of the ovipositor was brought slightly forward, as though to unlock it. It was then quickly moved posteriorly and resheathed. The typical post-oviposition action lasted from one to four minutes. It involved anterior-posterior motion of the first and second valvulae within the sheath and appeared to be similar to preoviposition movements. Due to the unfolding and refolding of the membrane attached to the lateral valvulae, it would appear that the post-oviposition action may help reposition these membranes. The female usually moved a short distance from the oviposition site and began to feed.
Substrates for Oviposition

The development of a chemically-defined substrate for oviposition was sought to use in experiments designed to identify plant components that might be inhibitory or stimulatory. In preliminary tests, females would not lay eggs in synthetic agar-bases substrates lacking natural plant material even though 3% sucrose was incorporated and feeding took place.

Oviposition responses to 3 synthetic substrates follow:

1. When a female was placed in the small observation cage in the presence of bean stem and a similarly shaped block of Parafilm-covered agar, she began to feed on the stem. After feeding on the stem for a time the female initiated typical preoviposition action. At this signal a piece of aluminum foil was gently moved to cover the bean stem and to force the female onto the Parafilm-covered agar. She proceeded to feed on the agar and continued the preoviposition action. After 1 to 2 min the preoviposition action stopped and did not begin again until the female was allowed access to the bean stem. In repeated trials she accomplished the typical preoviposition action while on the bean stem, but when forced onto the agar medium, the ovipositor was never unsheathed.

After the repeated trials, the female was finally allowed uninterrupted access to the stem but the ovipositor was not inserted into the plant and no egg was released. Even after 2 days the same female would initiate preoviposition action and unsheath the ovipositor, but she would neither insert the ovipositor, nor oviposit in the bean stem.

2. A female, caged on a Parafilm-covered matrix of thickened agar incorporating the fluids expressed from macerated bean-stem, fed almost constantly throughout the morning. In the afternoon she moved the
ovipositor within the sheath in a manner characteristic of preoviposition. The ovipositor was unsheathed but was not brought into contact with the substrate. After a few seconds the ovipositor was again resheathed. There was no further attempt by this female to lay an egg. Other females, subjected to the same test, proceeded with preoviposition action but did not unsheath the ovipositor.

3. Three females were individually caged on media similar to that above, but with the addition of the fibrous stem macerate. After 2 hr of nearly continuous feeding, the ovipositor of one female was unsheathed but was not brought into contact with the substrate. The ovipositor was resheathed but preoviposition movement continued for 7 min. The female continued to feed with no further preoviposition action or oviposition. The other 2 females displayed no action toward oviposition.

It was concluded from these three experiments that even though feeding requirements were met by the three substrates, at least one stimulus requisite to oviposition was lacking. Perhaps the surface quality did not provide information necessary to signal ovipositor penetration. The lacking factor may have been surface texture, or possibly gasses (e.g. O₂) normally emitted by living plant surfaces.

The behavioral pattern in ovipositing (and feeding as well) deviated from the typical pattern when the females were observed on Solanum chacoense. Relative to feeding on broadbean, the leafhopper's feeding on Solanum chacoense stem was sporadic. Often the female would leave the stem after feeding from 10 sec to 2 min. On an acceptable host, the adults fed almost continuously, defecating about every 30 sec which indicated some
measure of the liquid volume passing through the digestive tract. On the S. chacoense stem defecation was less frequent, occurring at intervals of 1 to 2 min.

While further investigations are necessary to clarify the relationships between feeding and ovipositing, it appeared that oviposition was regularly preceded by feeding. With reference to this feeding requirement, it would be interesting to learn if a fluid-filled gut is necessary to provide internal pressures requisite to the process of oviposition. If this were true, oviposition would be withheld while the female was not in contact with a host acceptable for feeding.

A further question is raised. Is it possible that food-energy requirements relative to the orderly process of oviposition demand frequent renewal prior to oviposition? The failure to oviposit after being held on the agar-thickened macerated-bean matrix may be evidence that although life-sustaining fluids were obtained, phloem-transported nutrients, necessary to meet the high energy requirements of egg production and oviposition, were too diluted. (The phloem feeding of E. fabae has been postulated by Lutman (1923).

Dahlman's (1965) findings, that alkaloids and alkaloidal glycosides (in certain concentrations) can influence (even completely restrict) imbibition, imply that oviposition may be indirectly prevented on plants bearing sufficiently high concentrations of those compounds. Refusal to feed may account for the refusal of E. fabae to oviposite on S. chacoense in my tests.
SUMMARY

In these observations of the mating pattern, the male produced sound as a part of premating behavior. The physical qualities of the sound (sound-I) produced by males were explored by using magnetic tape recordings and study of the oscillograms. To my knowledge this is the first reported recording of sound produced by *E. fabae*.

It appeared that sound-I may have served as a stimulus to mate-identification at close range, and possibly preconditioned the female for mating. Mate-identification seemed poorly reinforced since male-to-male contacts were frequent, male attempts to copulate with mating pairs were frequent, and female escapes were commonly observed. Sound-I is obviously a significant factor in the biology of *E. fabae*.

Male sound-II, which was similarly explored, was apparently not a part of the mating pattern, although it may play some role in territoriality. No sound produced by females was discovered.

Successful matings (i.e. yielding viable eggs) were accomplished by females during and after the third day in the adult stadium, and were recorded for females 120-days old. Females copulated as often as twice. Males, 2-days old were able to copulate, passing viable sperm, and were still sexually functional at 90 days of age. Males copulated with numerous females.

In the process of oviposition on *V. faba*, a readily accepted host, a typical behavioral pattern was observed. In this pattern manipulation of the ovipositor began within the sheath, continuing for as long as 30 min. During this action, feeding ultimately was discontinued when the female
advanced a few millimeters, unsheathed the ovipositor and began the process of cutting an egg cell within the plant. After about 2 min the ovipositor was withdrawn, leaving the egg in position, and the ovipositor was resheathed. Following this action, the female briefly manipulated the ovipositor within the sheath, advanced a few millimeters and resumed feeding.

Histological sections of females, stopped abruptly in the process of ovipositing, revealed an unexpected elongation of the egg in its passage through the constriction of the ovipositor and into the plant. At one point the egg extended from the genital chamber, throughout the length of the ovipositor, and partially protruded into the egg cell within the plant. This was made possible by deep convolutions of the chorion within the stricture of the ovipositor, and the chorion's flexibility. Release of the egg was accomplished by the ratchets of the second valvulae impelling it apically, and the ultimate separation and withdrawal of the ovipositor.

The typical pattern of oviposition on an accepted host was disrupted if females, preconditioned to oviposit, were confined to synthetic substrates or to Solanum chacoense even though they did feed. On synthetic substrates the typical pattern ensued to the point of unsheathing the ovipositor, but neither penetration nor egg release was accomplished. On S. chacoense the pattern broke at the initial feeding.

The role of plant-originating stimuli in influencing the processes of oviposition and feeding offers a natural phenomenon challenging further investigation.
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APPENDIX
Fig. 1 Rearing cage

Fig. 2 Observation cage
Fig. 3 Mating cage used in monitoring sound

Fig. 4 Equipment for recording and observing leafhoppers during sound production.

a. the large sound-proof box used in recording (right)

b. the smaller sound-proof box for recording while monitoring mating behavior (left)
Fig. 5 Oscillograms of premating sound (male sound-I)

a. Four phrases on a time scale of 0.1 sec (top)

b. Single phrase of ten complete pulses. Time scale, 0.1 sec (bottom)
Fig. 6 Oviposition cage

Fig. 7 Observation cage: technique for flooding with hot Duboscq fluid
Fig. 8 Cross section through genital regions of male (bottom) and female (top) in copulo. A., Aedeagus; E.C., Egg channel

Fig. 9 Sagital section through genital regions of male (bottom) and female (top) in copulo. F., Fluid (eosin positive); G.P., Genital plate; S., Sperm in spermatheca (inset)
Fig. 10  Leafhopper stance during ovipositor insertion

Fig. 11  Egg, E, positioned for release from the genital chamber to the ovipositor
Fig. 12 Leafhopper positioned to release the egg.

Fig. 13 Egg extended from the genital chamber, through the ovipositor, and into the egg cell within the plant. E., An extended egg.
Fig. 14 Lateral view of ovipositor. Vertical lines 15 (a,b), 16 (a,b), 17 (a,b), 18 (a,b) and 19 (a,b) indicate the approximate areas of cross sections. Cu.E., cutting edge of median valvula; L.V., lateral valvula; M.V., median valvula; T.G., tongue and groove coupling.

Fig. 15 Cross section of ovipositor (see Fig. 14 vertical line 15a,b).

a. T.G., tongue and groove mechanism; St.A., area stainable with alum hematoxylin; T.G.L.V., tongue and groove mechanism of the lateral valvulae

b. Portion of the egg within the genital chamber. Ch.E., chorion of the egg

Fig. 16 Cross section of ovipositor through fused area of the median valvulae

a. Ch.F., Chitinous flap; E.C., egg channel; F.A., fusion area

b. Flexible area of the lateral valvulae and convolutions of the chorion. E., egg; Fl.A., flexible area; T.A., toothed area
Fig. 17 Cross section of the ovipositor

a. St.A., alum hematoxylin stainable area

b. E., egg; Fl.A., flexible area distended during passage of the egg

Fig. 18 Median valvulae no longer fused dorsally (The tongue and groove mechanism between the lateral valvulae has terminated)

a. E.C., egg channel; Fl.A., flexible area

b. Egg chorion (Ch.E.) in contact with the toothed area (T.A.) of the median valvula. Fl.A., flexible area thin and membranous

Fig. 19 Cross section through distal end of the ovipositor

a. M.V., median valvula; T.A., toothed area of median valvulae

b. Egg separating ovipositor tips. L.V., lateral valvula
Fig. 20 Ovipositor halves separated by the passing egg; the distal ends of the ovipositor still intact

Fig. 21 Distal ovipositor halves separated upon release of the egg