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BIOLOGY OF THE CHICKEN BODY LOUSE, MENACANTHUS STRAMINEUS

by

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A Dissertation Submitted to the

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Major Subject: Entomology

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Dean of Graduate College

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INTRODUCTION

The majority of published work on the Mallophaga pertains to systematics, records of species from particular hosts and insecticidal control of those species infesting livestock and poultry. Although the chicken body louse, _Menacanthus stramineus_ (Nitzsch), is a common parasite of domestic chickens and turkeys throughout the world, very little is known of its biology.

Mallophaga spend their entire lives on the host and are extremely host and food specific. Most Mallophaga are difficult to keep alive once they have been removed from the host. Wilson (1928) sums up the difficulty of working with parasites by stating:

> "Probably no groups of living things offer greater difficulties to the student of science than do parasitic insects. The Mallophaga, or bird and mammal biting lice, stand as an example of this. At the present time there is no species of this order or suborder for which we have the complete life history."

The order Mallophaga is divided into the suborders Ischnocera, Amblycera and Rhynchophthiria. Only one species, found on elephants, comprises the Rhynochophthiria. The Ischnocera and Amblycera are found on both mammals and birds. In general, the members of the Ischnocera which live on birds are found on the feathers and their diet consists mainly of feathers. They maneuver well on feathers but are extremely awkward on smooth surfaces. This makes them easy to handle in the laboratory. Ash (1960) found that most ischnocerans are confined to particular areas of the host's body, and only rarely are found beyond the confines of these areas.

The Amblycera, or body lice, the group to which _M. stramineus_

belongs, appears to be dependent on some blood as food, in addition to feathers. Blood can clearly be seen in the crops, through the integument. Mallophaga have biting mouthparts and it is not clear how the liquid is obtained. These lice maneuver very well on flat surfaces, so are difficult to handle in the laboratory.

Since 1928, the life cycle of several species of feather inhabiting chewing lice, and two species of chewing lice of mammals, have been extensively studied in the laboratory. This successful laboratory rearing has not been possible with any of the body lice of birds, one of which is M. stramineus.

Although M. stramineus is a common ectoparasite of chickens and turkeys throughout the world, most of its basic biology is unknown. These investigations were undertaken to elucidate some of the biology of M. stramineus.

REVIEW OF LITERATURE

The names of the Mallophaga appearing in this paper are according

to the Checklist of Mallophaga by Hopkins and Clay (1952).

Very few reports appear in the literature regarding artificial rear-

ing of Amblycera. The first attempt at rearing Mallophaga off the host

dates back to P. H. Rolfs, a graduate student in biology (Osborn 1890). He

hatched the eggs of Dennyus dubius (Kellogg) (misdetermined by Osborn as

Nitzschia pulicare) a louse found on the chimney swift. Some of these eggs

were incubated using the heat from his own body and others were incubated

using a hen as a heat source. The louse eggs hatched between 5 and 20 days.

Attempts to rear Menopon gallinae (Linn.) and Menacanthus stramineus

by Wilson (1934) were unsuccessful. He used the same temperature and

relative humidity which were successful for Cuclotogaster heterographus

(N.).

By using a variable temperature apparatus, Conci (1952) found the

autoselected temperature for Menopon gallinae, the shaft louse of the

chicken, to be 39° C. Using the same apparatus, 42° C. was found to be

the preferred temperature for Menacanthus coronutus (Schommer) (=cornutus),

a louse sometimes reported from the chicken.

Bishopp (1942) states that the eggs of M. stramineus hatch in about

a week. Seventeen to 20 days are required for the newly hatched nymphs

to reach the adult stage, according to Van Es and Olney (1941), Bishopp

and Wood (1939) and Bishopp (1942). No experimental data are given to

support these statements, however.

Successful rearings under artificial conditions have been reported

for several species of ischoceran lice. Eight days was the shortest

incubation period for eggs of Philopterus citrinellae (Schrank)

(=Docophorus communis N.) taken from a red-winged blackbird, according

to Barber (1921). He used a temperature of 37° C. and states that

"moisture was supplied". His tests indicated that this species was not

light sensitive.

Four specimens of the chicken head louse, Cuclotogaster heterographus,

were reared by Wilson (1934) using an incubator temperature of 33 to 34°

C. He found the egg stage to be 5 to 7 days and the three instar stages

to be 6 to 14, 8 to 14 and 11 to 14 days, respectively. A total of 32 to

36 days were necessary to complete a cycle. The fluff portion of feathers

was used as food. Using this same louse, Ansari (1944) (he called it

Gallipeurus heterographus) found the complete life cycle to be 37 days

at 34° C. He fed a fresh feather once a day. By contrast, Bair (1950)

reported the autoselected temperature to be 44.49 ± 0.34° C. for C.

heterographus. He states that this compares favorably with the head and

neck temperature of the chicken which is 41.5° C. Bair did not control

humidity. Wilson et al. (1952), using a touch thermocouple, determined

the skin temperature of the breast surface, external and median femur of

a chicken to range between 37.8° C. and 39.4° C. when the room temperature

ranged between 17.7° C. and 32.8° C. When the room temperature was

raised above 32.8° C., the surface body temperature of the chicken rose.

Using the same technique he used for C. heterographus, Wilson (1939)

was able to rear three specimens of the wing louse of chickens, Lipeurus

caponis (Linn.) through to the adult stage in 22 to 34 days.

Lipeurus tropicalis (Peters), another ischnoceran found on chickens, primarily in India, was studied by Arora and Chopra (1957) at 35° C. and a relative humidity of 80 to 85 per cent. They reported the average life of the male and female to be 12 and 15 days respectively and the average number of eggs laid by each female to be 23. No mention was made of instar or life cycle length. Conci (1956) was not successful in rearing the large chicken louse, Goniodes gigas (Taschenberg) (=Stenocrotaphus gigas), using temperatures ranging from 35 to 40° C. and a relative humidity of 90 per cent or higher. Feathers were the one source of food.

Three specimens of the pigeon wing louse, Columbicola columbae (Linn.), were reared by Martin (1934) using an incubator temperature of 37° C. He did not report the relative humidity. The lice were placed on fresh feathers in an open petri dish. Stenram (1956) studied this same species and used an incubator temperature of 34.5° C. and 90 per cent relative humidity. Contrary to previous assumptions regarding the thermotactic responses of Mallophaga, Stenram found that this species was not attracted by the temperature of the host.

The cattle biting louse, Damalinia bovis (Linn.) (=Bovicola bovis) was reared through two generations by Matthysse (1946). He fed them cattle hair and brewer's yeast and found the optimum temperature to be 34.4° C. and the optimum relative humidity to be 70 to 84 per cent.

Scott (1952) in rearing the sheep body louse, Damalinia ovis (Schrank), found the optimum incubator temperature to be 36.5° C. at 70 to 90 per cent relative humidity, when a mixture of skin scurf and baker's yeast was used as food.

Matthysse (1946) successfully reared the slow moving cattle biting

louse, <u>Damilinia bovis</u>, by fastening cages on cattle.

Because of its rapid movement, <u>M</u>. <u>stramineus</u> does not lend itself

to confined rearing on the host. In preliminary investigations with this

species, lice quickly escaped from a cage attached to the back of a

chicken.

Removing living lice from host animals and birds in sufficient

quantities for laboratory use is time consuming. Injury is certain to

result to some lice if a forceps is used to pick them off. This is

especially true of fast moving Mallophaga such as <u>M</u>. <u>stramineus</u>.

Wilson (1928) obtained lice from a freshly killed bird by wrapping

it in cotton. As the bird cooled, the lice left it and became entangled

in the cotton. Lice were collected from live birds when Martin (1934)

clipped the feathers on which the lice were found. This method is

not suitable for collecting body lice because too few are found on

feathers.

A feather-by-feather examination was used by Ash (1960) in removing

ischnoceran species from living birds. His method of collecting the

fast-running amblycerans was:

> "by use of a pin-feather from a snipe, set in a wooden handle
> and moistened in alcohol. By parting the feathers and laying
> bare a portion of the skin of the host, the lice could be
> taken as they ran across."

Ash attempted to estimate the louse populations of birds by removing a

portion of the feathers where the particular louse species was found and

counting all the lice on these feathers. The number of lice obtained

from this sample count was multiplied by the average number of the

preferred feather on the bird, and the total louse population estimated. He states that the system was too erratic and range of variation was too great.

Gless (1957) picked the living lice off with a forceps but stated that it was easy to injure them using this method.

The defined diet of M. stramineus has not been ascertained. When examining the crop contents of various bird-inhabiting Mallophaga, Waterson (1926) found protective sheaths of growing feathers, feather fiber, down, skin, scurf, scales and cast skins of the lice.

Crutchfield and Hixon (1943) examined the crop contents of six species of poultry lice. Three of these, Goniodes gigas (=Goniocotes gigas), Goniocotes gallinae (DeGeer)(=Goniocotes hologaster), and Lipeurus caponis, were ischnocerans and contained only feather parts. The other three were all members of the suborder Amblycera, among which Menopon gallinae had only feather parts, while a Menacanthus sp. (probably third instars of M. stramineus) and M. stramineus contained blood, often in large quantities. Crutchfield and Hixon believed that the lice obtained the blood by rupturing quills, as well as by gnawing through the epidermis.

Kotlan (1923) found blood in the intestine of M. stramineus but thought its presence was accidental. A louse observed by Wilson (1933) had its mandibles deeply sunken in the quill of a young feather and blood in its alimentary tract. When the louse was removed, blood flowed from the wound. This circumstantial evidence indicates that blood makes up part of the diet of M. stramineus. In contrast, using morphology of the mouthparts as the basis for his reasoning, Barber (1921) claimed that

M. _stramineus_ is in no way adapted for securing blood and lives entirely

on feathers and scales of the hosts body.

Gless (1957) noted yellowish scabs were always present on birds

infested by M. _stramineus_. He thought they were formed from exudate

produced at breaks in the skin surface.

A study conducted by Waterhouse (1953) showed that, in addition to

blood, M. _stramineus_ had feather particles up to 800 μ in length in its

crop. He made no mention of differences in the crop contents of nymphal

instars compared with adults. While rearing the pigeon wing louse,

Columbicola columbae, Martin (1934) found that feather barbules made up

the entire diet.

A louse, _Lipeurus tropicalis_, from the chicken was found by Arora

and Chopra (1957) to feed on feather barbs and barbules, loose scurf,

pellicle of the skin, sheaths of the growing feathers and coagulated

blood that had oozed out of the body of the host. Wilson (1934) found

that the chicken head louse, _Cuclotogaster heterographus_, depends on

feathers for its food supply but supplements this with blood when it is

obtainable. The pigeon wing louse, _Columbicola columbae_, was found by

Martin (1934) to feed entirely on feather barbules obtained primarily

near the proximal end of the feather.

All species of Mallophaga which have been reared _in vitro_ have

three nymphal instars (Martin, 1934, Wilson, 1934, Matthysse, 1946,

Scott, 1952 and Arora and Chopra, 1957). The descriptions of most

Mallophaga only include information on the adults. Exceptions to this

are Ansari's (1944) description of the nymphs of _Cuclotogaster hetero-_

graphus, Martin's (1934) description and measurements of nymphs of

Columbicola columbae, Wilson's (1939) description of the nymphs of
Lipeurus caponis, and Matthysse's (1946) measurements of Damalinia bovis.
Measurements or descriptions of nymphs of M. stramineus are not in the
literature, although Gless (1957) shows a colored picture of what he
thought was four nymphal instars and a male and female.

Using the shaft louse, Menopon gallinae, Hoyle (1938) was able to
infest the sparrow. He placed two chickens infested with M. gallinae
in a cage with five parisite-free sparrows. One month later, careful
examination of the sparrows revealed a half-grown louse and four first
instars, plus the empty egg cases. In a later similar experiment, 16
per cent of the sparrows became infested with M. gallinae.

The possibility of a louse species establishing itself on two
unrelated hosts is extremely remote. Owls, and other birds of prey,
provide an excellent opportunity for the establishment of parasites of
their prey upon themselves. Baker (1931) states that he knows of no
record of the establishment of a species from a passerine bird. He also
states that the cuckoo has never been recorded to have lice of any of
its foster parents but only cuckoo-infesting species. He believes the
chemical composition of blood, skin and plumage of the unnatural host is
such that its body does not provide an attractive source of food and
shelter, and may actually be lethal to the straggling parasite.

Dissemination of M. stramineus has remained a mystery. Dispersion
probably occurs when infested birds are housed or mix with non-infested
birds. Midwestern turkey flocks occasionally become infested with M.
stramineus and the mechanism of this infestation is not clear.

Closely related birds, such as pheasants and chickens, could con-

ceivably share the same species of lice. This could make the ring neck

pheasant, Phasianus colchicus Linn., a means of dissemination of M.

stramineus to range chickens and turkey flocks, since the pheasant may

frequent or mix sparingly with chickens and turkeys on range. The

literature reports that the chicken body louse often infests pheasants

in captivity (Piaget, 1880, p. 469 and Mönnig, 1938, p. 330).

Phoresy is a method of dissemination used by certain Mallophaga.
Smit (1953) and Thompson (1934) found a badger louse, Trichodetes melis

(Fab.), attached by its mandibles to the hind tibia of the badger flea,

Chaetopsylla trichosa Kohaut.

Hippoboscid flies are often collected with Mallophaga clinging to

their bodies (Thompson, 1936, 1947; Clay and Meinertzhagen, 1943; Ansari,

1946; Ash, 1952; Bequaert, 1953; and Corbet, 1956).

Since midwestern poultry are not infested with hippoboscid flies or

fleas, these insects would not be a factor in disseminating M. stramineus.

However, mosquitoes or other species of flies could conceivably carry M.

stramineus from one poultry flock to another.

Sparrows are often found in poultry establishments. Although the

chances of a body louse traveling from a chicken to a sparrow are rather

remote, it still could happen. In addition, the sparrow, carrying the

straggling parasite, would need to travel to the new flock before the

"hitchhiking" body louse could safely leave to infest a chicken.

Detailed descriptions of the copulatory act are recorded for several

species of ichnoceran lice found on birds (Martin, 1934; Wilson, 1934;

Stenram, 1956; and Arora and Chopra, 1957). Wilson (1934) kept males

and females of Cuclotogaster heterographus separated for a time, and

when he placed a male and female together, they mated readily. The male

assumed a position ventral to the female, and bent the tip of his abdomen

dorsally to come in contact with the posterior tip of the female. At

the same time the male gripped the female with the enlarged segments of

his antennae. Martin (1934) observed that coition lasted from 10 to

75 minutes for the pigeon wing louse, Columbicola columbae.

Although Wilson (1934) did not observe the complete molting process

for Cuclotogaster heterographus, he observed specimens which had the

entire body out of the exuviae except for the anterior tip of the head.

This indicates that removal of the head is the last step in the molting

process.

Martin (1934) reported that 31 female Columbicola columbae lice laid

a daily average of 0.52, 0.33 and 0.31 eggs per female under laboratory

conditions for three laying periods consisting of five, six and six days

respectively. Three to five eggs may be laid each day by Lipeurus

tropicalis, according to Arora and Chopra (1957). They state that females

lay an average of 23 eggs, and adults live for 12 to 15 days. Oviposition

starts two to three days after copulation and requires two to three

minutes per egg.

Wilson (1939) reported that the chicken wing louse, Lipeurus caponis,

may lay as many as 35 eggs under laboratory conditions. One female laid

fertile eggs for 30 days following the death of the male. This indicates

that one mating may be sufficient for fertility.

Ansari (1944) reported that Cuclotogaster heterographus laid 14 to

26 eggs during a 15- to 20-day period when reared in captivity. The

maximum number of eggs per day was three, with 20 to 25 per cent of the

eggs infertile. The first and last batches of eggs during the oviposition cycle often were infertile.

Martin (1934) watched several eggs of <u>Columbicola</u> <u>columbae</u> hatch. She states that the process begins with the nymph pumping air into its body through the mouth. Air bubbles accumulate in the digestive tract until pressure forces them into the egg shell at the posterior end. After about five minutes, the pressure behind the nymph causes the operculum to burst open. She states that the embryo's head is folded against the ventral aspect of the thorax and that the whole hatching process requires about 20 minutes. This explanation of eclosion is contrary to that given by Sikes and Wigglesworth (1931) in their very excellent article on the eclosion of fleas, mealworms and Anoplura. They have never observed the swallowed air or fluid to be forced out the anus. This swallowed air is used to force the body fluids forward causing the pressure which breaks the operculum.

Ansari (1944) states that the chicken head louse also sucks air through the operculum prior to its opening. He states that the hatching process takes 40 to 60 minutes.

IN VITRO REARINGS

Materials and Methods

When a feather was removed from a checken infested with M. stramineus, lice would often come to the injured area and feed on the fluids oozing from the wound. If blood was present, it would soon show through the transparent exoskeleton. Because of this observation, a series of trials was made in an effort to keep this louse alive off the host in the laboratory.

Source of adult lice

Laying hens, obtained from a local poultry processing plant, were maintained as the source of lice for these investigations. These hens were kept in wire-floored pens in a small insulated building close to the Insectary.

Lice were removed from the chickens with the aid of a motor-driven aspirator (Fig. 1). Several hundred adult lice could be obtained by this method in a few minutes from a heavily infested host bird. The vial containing the lice was immediately brought into the laboratory and its contents placed in one-half of a large petri dish. During preliminary investigations an electric water-heating cable was wrapped around a large petri dish to form a heat barrier to keep the lice in the exposed dish. From this they could be transferred to rearing containers or to chickens being infested for test. Many lice succumbed to the high temperatures near the heat barrier instead of moving to the cooler middle region of the petri dish. Because too many lice were lost, this

Fig. 1. Motor-driven aspirator used for removing adult M. stramineus from chickens.

A. Vacuum pump

B. Electric 1/4-horse motor

C. Collecting vial

D. Collection tube

Fig. 2. Plastic zipper boxes lined with silk bolting cloth used in the in vitro rearing of M. stramineus

Fig. 3. Water bath used as an incubator for in vitro rearings, with lid removed

Fig. 4. Water bath used as an incubator for in vitro rearings
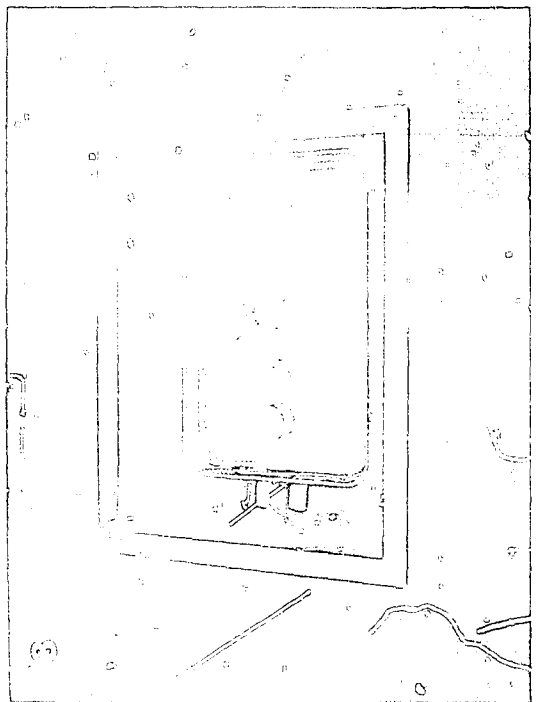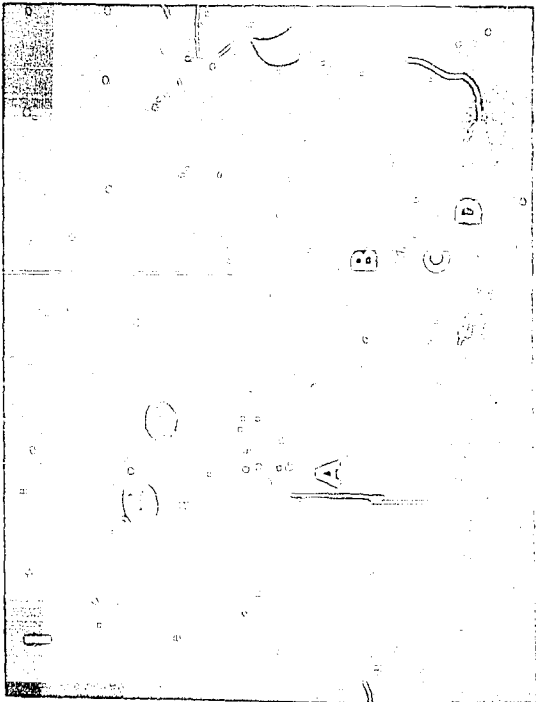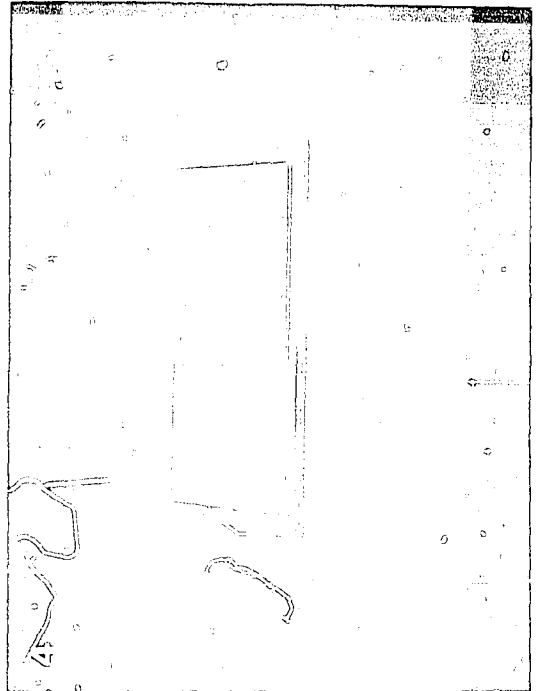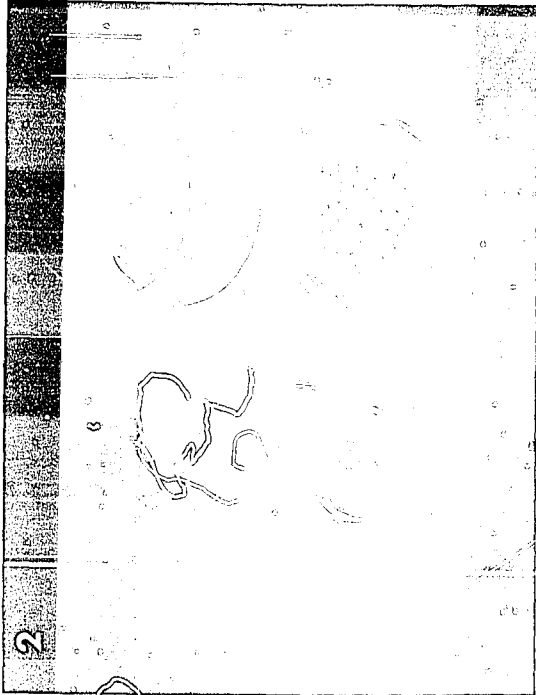
method of restricting them was abandoned in favor of using cool temperatures.

The lice were sorted to sex, counted and placed in the desired cages, all within a walkin cooler operating at 3.3° C. At this temperature the lice quickly became sluggish and very easy to sort into cages. A small camel's hair brush was used to transfer the lice from one container to another.

Louse cages

The cages (Fig. 2) used in all of the in vitro investigations were of two sizes of round plastic zipper-boxes obtained from Coats and Clark.[1] The larger was 5 cm in diameter and 2.6 cm deep. The smaller was 3.1 cm in diameter and 2.6 cm deep. Ten to 20 holes approximately 2 mm in diameter were made in the top and bottom of the cages by plunging a heated probe through the plastic. Silk bolting-cloth was used to line the top and bottom of the containers to prevent the lice from escaping through the ventilation holes. A casein adhesive held the cloth in the plastic cage. The tight fitting friction cover prevented louse escape and was easily removed.

Sources of heat

Incubators made by the Chicago Surgical and Electrical Company[2] were used as a source of heat for a portion of these studies. These incubators held the temperature within ± 1° C. Various relative humidities were

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[1] Coats and Clark, Warren, R. I.

[2] Chicago Surgical and Electrical Co., Chicago, Ill.

maintained in these ovens by exposing different amounts of water surface

for evaporation. The louse cages were placed on metal shelves four to

six inches above the water surface. These shelves had holes one-half

inch in diameter, and each cage was always centered over one of these

holes to aid in ventilation.

A water bath[1] (Figs. 3 and 4) was also used as an improvised incu-

bator. An aluminum cake-pan, 11-1/2 by 8-1/2 inches was floated on the

water surface. Plastic cages containing the lice were placed in this

aluminum pan. This constant temperature source was used for the majority

of the tests. The successful rearing of lice in the water bath was due

to the extremely high relative humidity which provided a favorable environ-

ment to the lice and maintained the high water content of the feathers

used as food.

A slide dryer[2] (Fig. 5) was also used as a heat source during por-

tions of these investigations. It was maintained in the laboratory at

room humidity. When cages of lice were removed from the incubators for

feeding, counting, and data recording, they were placed on this slide

dryer to keep them at the desired temperatures. This aided in keeping

the lice at the bottom of the cage near the source of heat, and very

little difficulty was experienced with the lice escaping when the container

lid was removed.

Adult lice, removed from the chicken, were maintained in the labora-

tory at temperatures of 30, 32, 34, 36 and 38° C. With relative humidities

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[1]Precision Scientific Co., Chicago, Ill.

[2]Chicago Surgical and Electrical Co., Chicago, Ill.

of 40 to 95 per cent. Relative humidity was measured with a battery-operated psychrometer.[1]

Food source

Because lice were observed feeding on the liquid portion of the wound caused by removal of a feather, freshly plucked pinfeathers (Fig. 6) were placed in the cages as food. These fresh feathers, the shafts of which were filled with a pulpy, liquid material (lymph and blood), were placed in the containers at various intervals during a 24-hour period. Young broiler chickens furnished the pinfeathers during the course of the investigations.

Source of first instar nymphs

Several eggs, laid by lice in rearing cages, hatched and the resulting nymphs were reared to adults. Since much more biological information could be obtained if recently hatched first instars were observed, all subsequent in vitro studies began with nymphs a few hours old.

Louse eggs were obtained by plucking a feather, containing the mass of eggs (Fig. 7), from a bird heavily infested with lice. The feather was placed in the water-bath incubator at 35° C. and 95 per cent relative humidity. Several hours prior to starting a test, nits were examined under a binocular dissecting microscope and all newly hatched lice were destroyed. Later, after a sufficient number of nymphs had hatched, they were placed in the smaller plastic rearing containers. Thus, each container held 6 to 10 lice of a known age. The containers were placed in the

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[1]The Bendix Corporation, Friez Instrument Division, Baltimore, Md.

Fig. 5. Slide dryer used as
a source of heat dur-
ing portions of the
in vitro rearings

Fig. 6. Several types of pinfeathers
which were used as food for
in vitro rearing of nymphs
and adults

A,B,C and D. Examples of pinfeathers
which successfully furnished
the food requirements for M.
stramineus

E. Basal (1/4-inch) feather tip
which was successful in rear-
ing several M. stramineus

F. Fluff feather which would
not sustain lice when it
was the only food

Fig. 7. Mass of louse eggs
at base of feather

Fig. 8. Separatory funnel used in
washing lice from birds
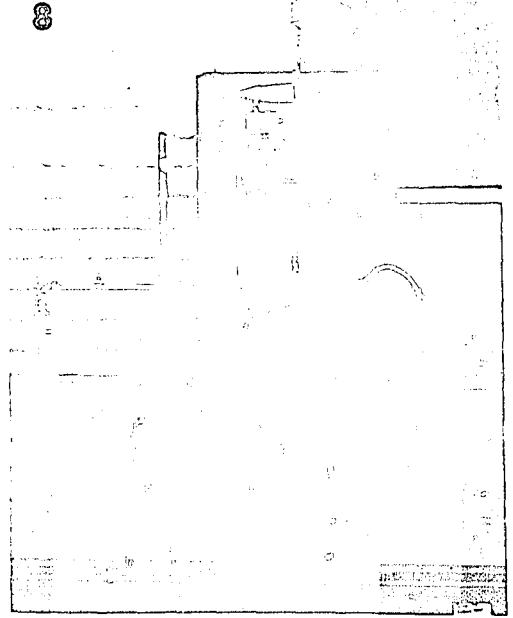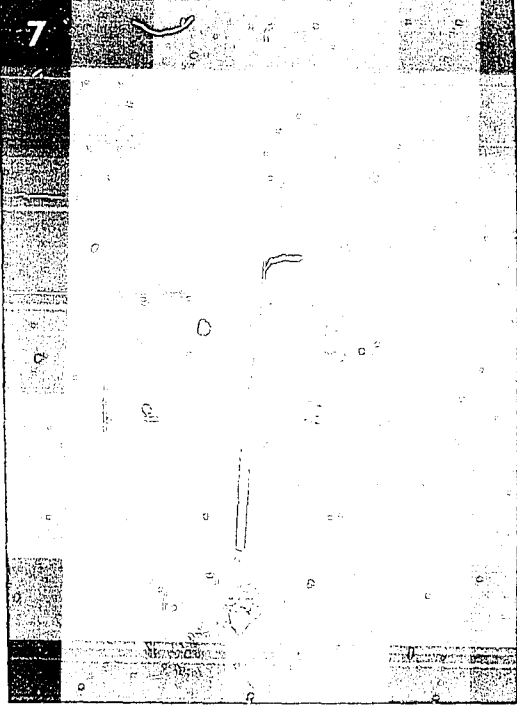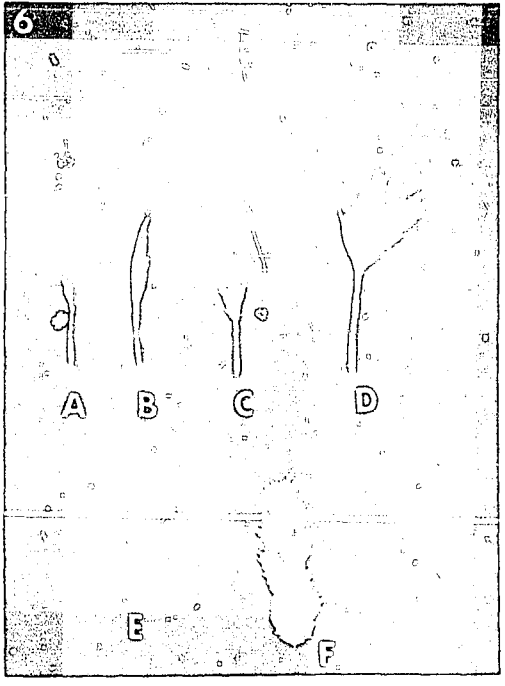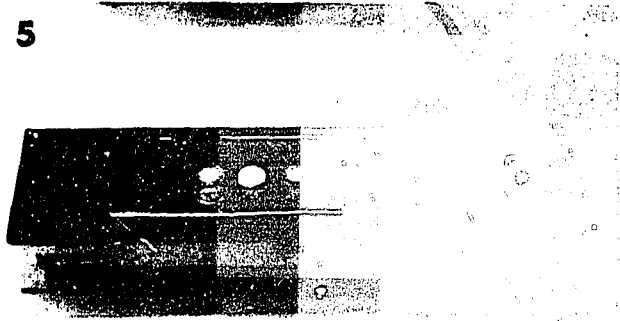
incubators with various temperatures and relative humidities. The lice

were fed a freshly plucked pinfeather, at three- to four-hour intervals,

seven times during a 24-hour period. Once during each 24-hour period the

lice were examined. The number surviving, and their progress toward

maturity, were recorded. At this time all accumulated feathers from the

previous 24 hours were removed. Later experiments were successfully

conducted when fresh pinfeathers were fed every six hours (four times a

day).

Observations and Discussion

Rearing Adults

Test 1. At 8 PM Sept. 15, a motor-driven aspirator was used to

remove 14 adult M. stramineus from a chicken. These lice were placed in

a 5 cm diameter plastic cage which was placed on a slide dryer operating

at 35° C. The slide dryer was on a laboratory bench and no attempt was

made to regulate the humidity. Twelve hours later a fresh feather was

placed in the incubator. Fresh pinfeathers were added at 3-hour intervals

until 10 PM. This feeding cycle was repeated each day until the termina-

tion of the test.

After the first 12 hours the lice stopped their apparently aimless

wandering and remained on the bottom of the cage. They made no attempt

to escape when the lid was removed to feed them. As soon as the fresh

pinfeather was added, several lice would approach the feather tip and

immediately feed on the liquid portion. One louse was kept alive for 10

days. The death rate of these 14 lice is shown in Fig. 9.

This preliminary test indicated that the fresh pinfeather was an

important source of food for M. stramineus. Another important aspect, revealed by this test, dealt with movement of lice. When the lice were first placed in the cage they moved rapidly about on the top, sides and bottom. After a few hours, they seemed to become adjusted to their new surroundings. Their movements were slower and they remained on the bottom of the cage. This "adjustment" may have been an important factor in the length of time that the lice were kept alive off of a host.

Several additional tests were conducted in an attempt to verify the results of Test 1. These were not successful. Although most conditions were the same, Test 1 was conducted in September, before the room radiators were on. The relative humidity of the laboratory was considerably higher than at later dates in the fall when the room was heated. Perhaps the lower relative humidity was the limiting factor in follow-up attempts to maintain M. stramineus off the host for as long as 10 days.

These tests all had the long interval between feedings at 11 PM and 8 AM the next day.

Test 2. In order that the variable of irregular feedings could be eliminated, a vigorous 3-hour interval feeding schedule around the clock was adopted. To determine if humidity was a factor, a second slide dryer was placed in a rearing room with a relative humidity of 66 to 70 per cent. Five adult lice were placed in each of four containers. Two of the containers were placed on the slide driers in the room. The remaining two containers were placed on the slide dryer in the laboratory where the room relative humidity was 40 per cent or below. The slide driers were set at 35° C. The room temperature varied between 21 and 27° C. in both cases.

Fig. 9.   Survival curve of M. stramineus incubated at 35° C. and
          room relative humidity.   Test 1

Fig. 10. Survival curve of M. stramineus incubated at 35° C. and
          relative humidities of 40 and 66-70 per cent.   Test 2
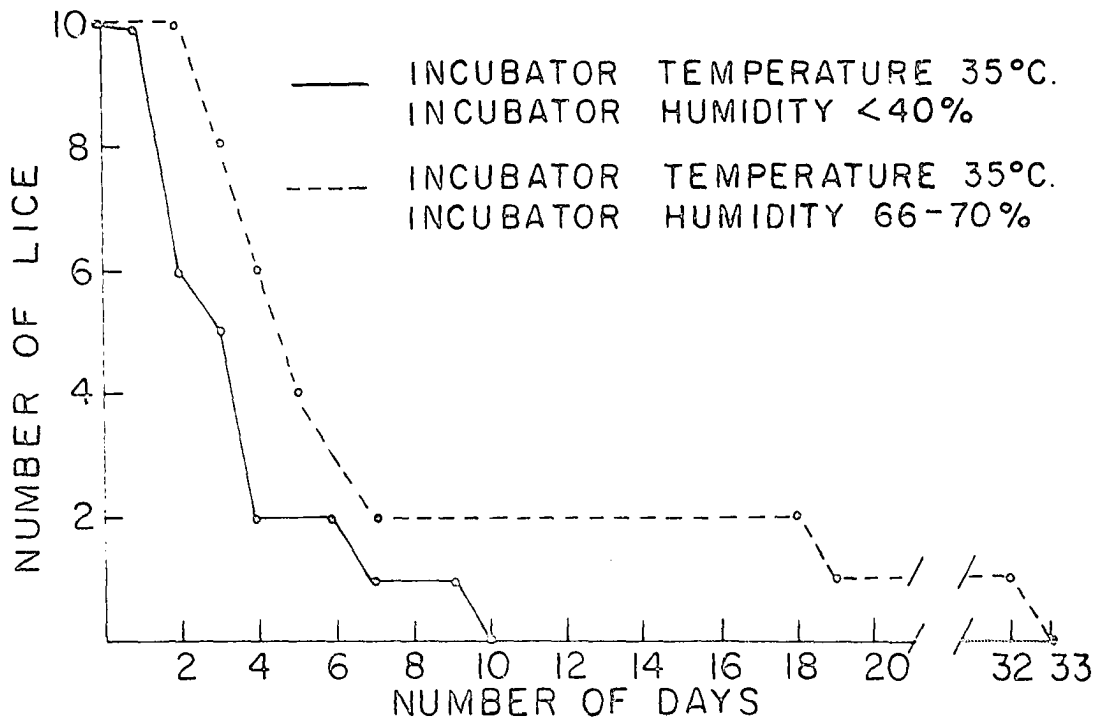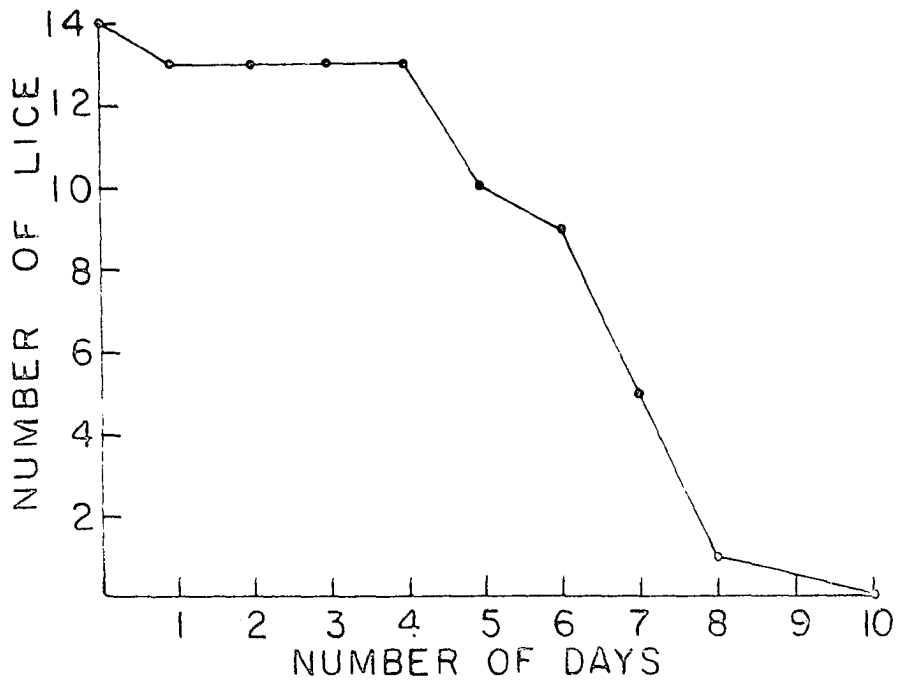
Fig. 10 shows the death rate for the two groups of lice. During the first three days, over 50 per cent of the lice died in both locations. One louse lived for 10 days at the lower humidity. At the higher humidity the last louse succumbed after living 33 days. No conclusions could be made regarding the vigorous 3-hour interval feeding schedule.

Test 3. For this test, 128 adult lice were removed from an infested bird. Eight lice were placed in each container. Five of these containers were placed on a slide dryer in a room with the temperature at 27° C. and the relative humidity at 40 per cent. Another 40 lice in five containers were placed on a slide dryer in a room with a temperature of 25° C. and relative humidity of 70 per cent. The third group of 40 lice were placed in an incubator with a relative humidity of 76 per cent. This humidity was maintained by exposing 200 square inches of water surface in the 8,424 cubic-inch capacity incubator. All units were set at 35° C. A pinfeather was placed in four of each group of five containers every three hours around the clock, or eight times in a 24-hour period. Starting the tenth day, feeding periods were reduced to seven during a 24-hour period instead of eight. The lice in the fifth container at each of the three stations were not fed. Once every 24 hours, the lice were counted and the accumulated feathers were removed from the containers.

In all three locations the unfed lice were dead within 21 hours. Fig. 11 and Table 7 show that very little difference existed in the mortality of fed lice living under the three different conditions. The highest death rate occurred during the first three days in all three locations, which indicates that louse adjustment to the incubator conditions may be an important factor when rearing M. stramineus in vitro. The

energy wasted with rapid futile wanderings in the cage undoubtedly

contributes to the death loss in the early days of test. No differences

in louse survival due to variations in humidity could be noted from this

test.

Test 4. In addition to the three locations utilized in Test 3, a

hot water bath was used in Test 4. The cages containing the lice were

placed in an aluminum pan which floated on the water. The water tempera-

ture was 38° C. and the air temperature at cage height was 35° C. The

relative humidity was 95 per cent. Four cages, each containing eight adult

lice, were placed in each of the four locations. No control group of

unfed lice was started in this test because of the uniform death loss of

lice during the first day in Test 3. Fig. 12 and Table 8 show that the

higher humidities offered by the water bath and the rearing room appeared

to be favorable for louse survival. This test was terminated after 14

days.

These tests showed that the pinfeather was furnishing the nutritional

requirements for the lice to survive. The percentage of lice which died

during the first two days after removal from the host was quite high.

The rapid aimless wandering while the lice adjusted to the new environment

undoubtedly contributes to the high mortality during the first days of

the tests. The subsequent death rates were comparatively low.

A handicap in this type of observation was the unknown age of the

lice. Theoretically, a percentage of lice would normally die during a

given period even though they remain on the host. Keeping one louse

alive for 34 days in Test 2 indicates that its nutritional and environ-

mental needs were nearly being met.

Fig. 11. Survival curves of M. stramineus incubated at 35° C. and
          relative humidities of 40, 66-70, 70 and 95 per cent.
          Test 3
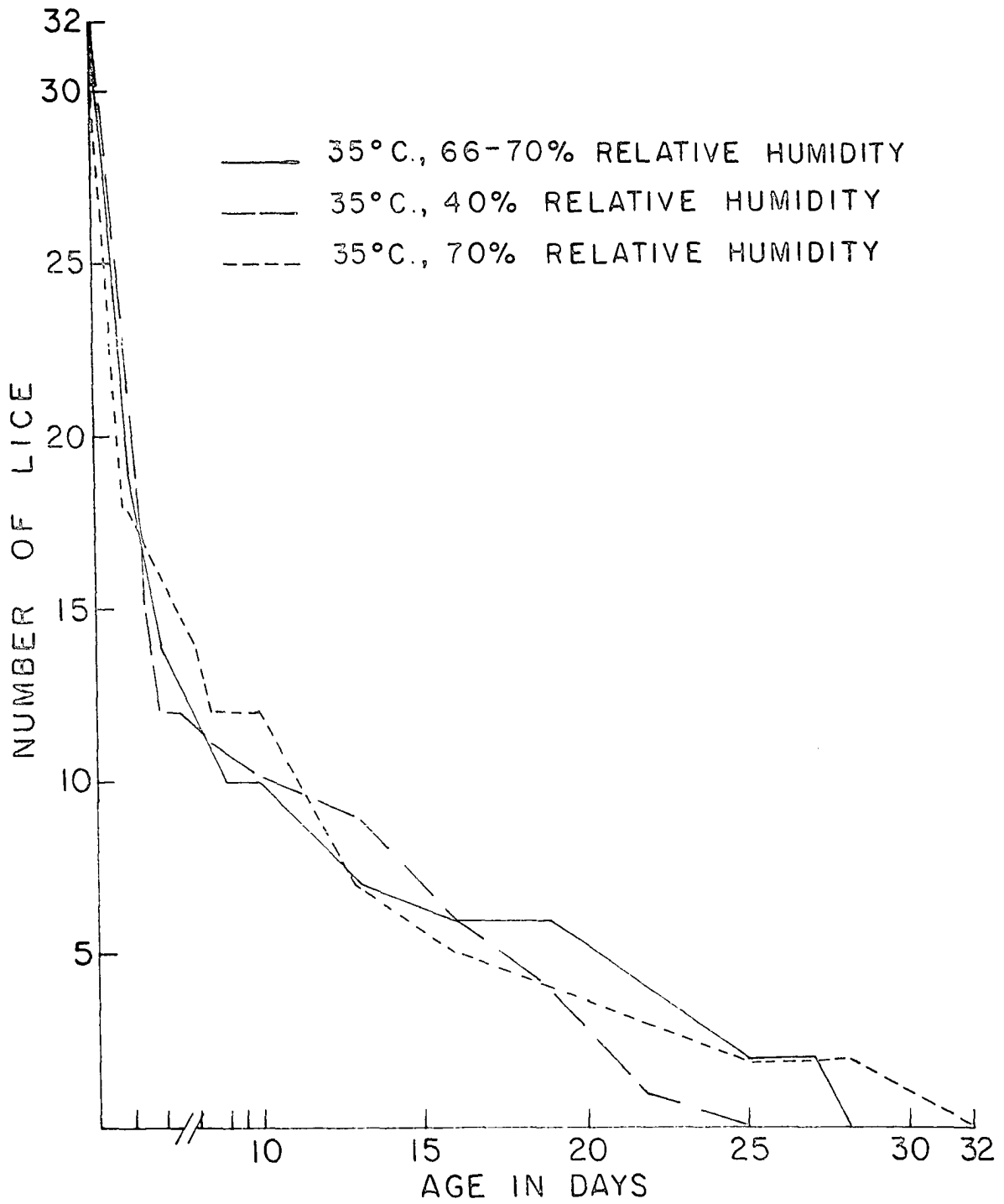
Fig. 12. Survival curves of <u>M</u>. <u>stramineus</u> incubated at 35° C. and
relative humidities of 40, 66-70 and 70 per cent. Test 3

35°C., 95% RELATIVE HUMIDITY
35°C., 70% RELATIVE HUMIDITY
35°C., 40% RELATIVE HUMIDITY
35°C., 66-70% RELATIVE HUMIDITY
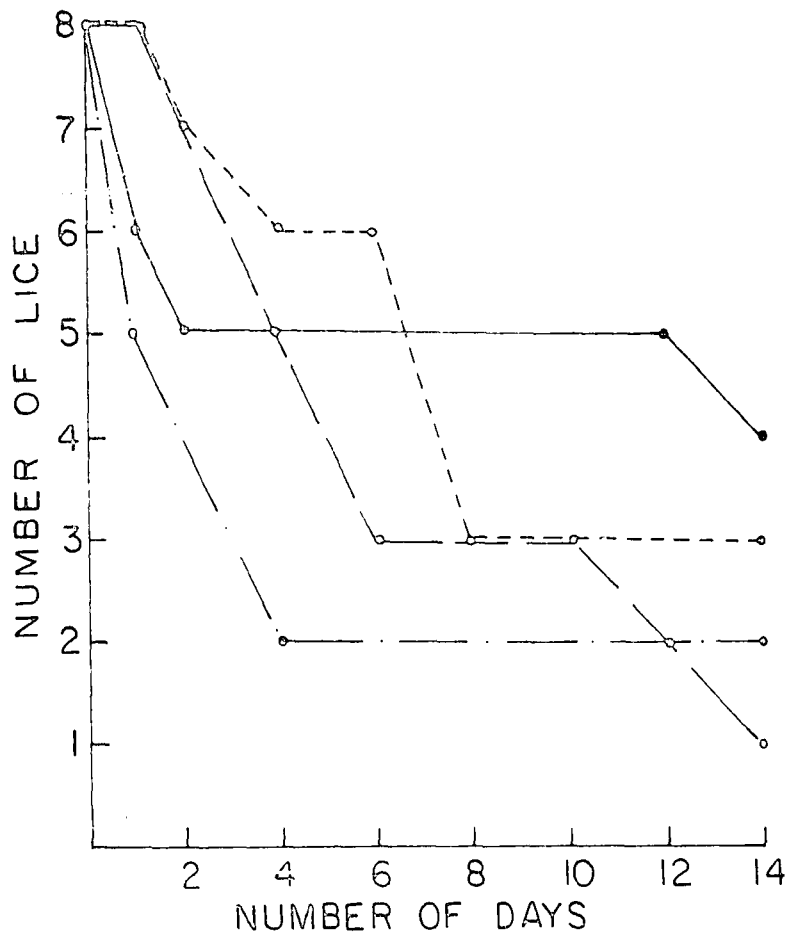
Rearing first instars

As soon as it was noted that nymphs hatching from eggs laid by females in Tests 1, 2 and 3 were successfully reaching the adult stage, several tests were initiated using recently hatched first instars. Sufficient numbers of first instars were readily available using the technique described on page 18. All of the lice were fed freshly plucked pinfeathers at 3- to 4-hour intervals (7- to 8-times a day). Again, once each day the accumulated feathers were removed and survival and growth data recorded.

Several different combinations of temperature and humidity were used in the rearing tests. The number of days required to reach the adult stage and the percentage of lice reared to maturity were the two criteria used to determine the effectiveness of the various environmental conditions.

Table 1 shows the results of tests conducted during a two-month period. The highest percentage of lice were reared from the egg stage to maturity in the shortest time by using the water bath set at 35° C. and 95 per cent relative humidity. Nine to 13 days were required to advance from hatching to the adult stage with 59 per cent of the lice reaching maturity. The 3-day interval appeared to be the minimum period required for each instar.

None of the other combinations of temperature and humidity were as beneficial for lice, but in several combinations a few lice reached the adult stage in 9 days. A temperature of 30° C. was too low. No lice were reared beyond the second instar at this temperature. Lice did not survive beyond the first instar under the conditions of a slide dryer

Table 1. Results of rearing first instars of M. stramineus to adults at several combinations of temperature and humidity

Date started	Heat source	Temp.	% RH	No. days in each stage			Hatching to adult stage	No. of specimens reared	% reared
				1st instar	2nd instar	3rd instar			
4/15	Incu-bator	30°C	70	6-7	4	All died			
4/11	"	32	70	5	3-5	3-6	13-15	2/16	12.5
4/15, 16	"	34	72	3-4	3-4	3-5	9-15	9/37	24.0
4/8	Slide dryer	34	40-60	None					
4/3	"	34	70	3-5	3-4	3-7	11-16	8/50	16.0
4/8, 10, 11, 15, 16	Water bath	35	95.0	3-4	3	3-6	9/13	55/93	59.0
3/14	Incu-bator	36	76	4	4	4	12	1/5	20
4/8, 11, 15, 16	"	38	70	3	3-4	3-4	9	14/64	21.5

operating in low room humidities. The same heat source at 70 per cent

relative humidity provided conditions in which 16 per cent of the lice

matured in 11 to 16 days (Table 1).

The tests showed that a temperature of 34 to 35° C. was nearly

optimum.

IN VIVO REARINGS

Materials and Methods

Because this louse escaped from confining cages on a chicken, the biology of M. stramineus was studied by infesting louse-free birds with a known population of lice nearly the same age. By sacrificing these artificially infested birds at regular intervals and recovering their lice, biological information was obtained.

Debeaked broiler chicks were infested with adult lice in Test 5 while the chicks in Tests 6 and 7 were infested with recently hatched first instars. The lice were obtained as described in the in vitro tests, (adults, page 13, first instars, page 18). A camel's-hair brush was used to place lice on the chicks. The chicks were not isolated from each other but were isolated from possible louse contamination from other sources. They were sacrificed at the intervals indicated and their lice removed using a washing technique. Lice were mounted in PVA plus lacto-phenol.[1] Head capsule measurements were taken using an ocular micrometer in a binocular microscope at 100x magnification.

Washing technique

The chick was killed by a blow on the head and immediately placed in a 2- or 4-quart, wide-mouthed jar. A few drops of a liquid detergent was placed in the jar to serve as a wetting agent to help free the lice from the feathers. Enough warm water was then added to make the jar one-half to three-fourths full. The chick was shaken vigorously in the

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[1]Formula for the media is given in the Appendix under Mounting Media.

tightly sealed jar for three to five minutes. The contents were poured

into a separatory funnel (Fig. 8). The jar was rinsed with tap water and

these rinsings were poured over the chick as it was held by the head over

the funnel. A few milliliters of 70 per cent alcohol was placed on the top

of the water in the separatory funnel to eliminate the suds to which some

of the lice would cling. Gently stirring the contents aided in removing

any suds remaining on the water surface, and helped settle the remaining

lice.

In Test 7, 4 to 5 ml of a solution of 50 per cent Aerosol OT[1] in

alcohol were used instead of the liquid detergent. This created very

little suds and was an effective wetting agent. Although both wetting

agents were effective in freeing the lice from the chicks, the Aerosol-

alcohol solution was more dependable because no lice would cling to parti-

cles on the surface of the contents in the separatory funnel. In prelim-

inary tests with this technique, 100 per cent of the lice which had been

placed on the birds a few hours before washing, were recovered.

The washings were allowed to settle in the separatory funnel for

10 to 15 minutes before 50 ml of water containing the lice were removed

from the bottom of the separatory funnel. The lice were then picked from

the wash water while being viewed through a dissecting microscope.

Observations and Discussion

Rearing adults

Test 5.   To study the rate of increase of a louse infestation, 54

debeaked cockerals, 2 days old, were infested with five adult lice each.

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[1]Fisher Scientific Co., Fairlawn, New Jersey.

Table 2. Average number of lice of each instar recovered from washing two chicks at intervals after five adult M. stramineus were placed on the birds

Stage	Days after infestation								
	3	5	7	10	12	14	16	18	20
1st instar		.5	5.5	29	23.5	20			
2nd instar				23.5	21.5	12.5			
3rd instar					11.5	12.5			
Adults	4	4.5	8	4	5	3	13	14	20

Previous counts of adult lice revealed a 50:50 ratio of males to females, so no attempt was made to sex the lice. Infested chicks were not isolated from each other.

One first instar nymph was recovered five days following infestation of the chicks (Table 2). This would indicate that egg laying took place soon after the infestation. The table shows that each of the three instar stages require about three days, and the total period from egg to adult is 15 to 16 days. Since it was impossible to ascertain how soon after infestation oviposition began, in subsequent tests the birds were infested with first instar lice all within a few hours of the same age.

Test 6. Six first instar nymphs were placed on fourteen, 3-day old chicks. The chicks were not isolated from each other. The first washing was made two days after the infestation primarily to check the "take of the infestation" and the effectiveness of the washing technique.

Table 3 shows the number of lice recovered from the washings at various intervals after the infestation. Although the numbers of lice

Table 3. Lice recovered from chicks infested with six newly hatched
1st instar nymphs of M. stramineus[a]

No. of days after infestation	No. of chicks washed	Louse stage				
		1	2	3	Female	Male
2	1	5				
5	2	1	5			
7	2		1	2		
8	3			3		
10	2				1	2
11	1					
14	2				2^b	
17	1					
19	2				1	

[a]The percentage of lice recovered in this test was 24 per cent.

[b]Eggs were clearly visible in the abdomen of both females.

recovered in this test are few, the data indicate a requirement of three days for each of the three nymphal instars.

Test 7.   In an effort to obtain more data to substantiate the number of lice recovered in Test 6, the recovery test was again repeated. Six first instars each were placed on 8-day old debeaked broilers. The 22 chicks were again infested with nymphs less than 12 hours old at the time of infestation.

Again a 3-day interval for each instar was observed (Table 4). A 39.5 per cent louse recovery was made for the chicks washed during the

Table 4. Lice recovered from chicks infested with six recently hatched 1st instar nymphs of M. stramineus

No. of days after infestation	No. of chicks washed	Louse stage				
		1	2	3	Female	Male
1	1	6[a]				
3	2	3				
4	2		3			
5	1		3			
6	1		2	1		
7	2		1	4		
8	2			6		
9	2			3		
10	1					2
11	2				2	2
19	1					
20	4					
22	1	1	7		1	
24	1		5	5		
26	2				1	

[a]This washing was made to test the effectiveness of the recovery techniques.

first 11 days of the test. The first washing, one day following the infestation, recovered 100 per cent of the lice. On the sixth and seventh days after infestation, a slight overlap in second and third instars existed.

Since only adults were recovered on the tenth and eleventh days, an 8-day interval was allowed for the remaining lice to mature, mate and oviposite, and the eggs to hatch before the remaining chicks were washed. Table 4 indicates that the adult life span on young chicks may be short since only two adult second generation lice were recovered during this same period. In vitro egg laying data reported later in this work show that the peak oviposition period takes place when the adults are five to six days old. Many insects succumb shortly after their oviposition is completed. This mortality could be expected also with M. stramineus.

The method of infesting louse-free birds with lice of a known age proved to be quite satisfactory in determining the time interval for each instar. In these tests the assumption was made that the baby chick would furnish environmental conditions similar to an adult bird. The minimum time-interval for the three instars was found to be three days in the in vivo rearing investigations. The number of lice, initially placed on a bird and recovered with the washing technique, decreased as the interval between infestation and washing increased. Table 4 shows that 39.5 per cent of the lice which were placed on the chicks were recovered for the first 11 days of Test 7. Since the louse recovery was always nearly 100 per cent when washings were made soon after infesting the chicks, it is certain that natural mortality does occur on the host. At molting time, insects are quite susceptible to destruction. During, and immediately following molting, the exoskeleton is soft and does not offer protection to the insect. Ectoparasites such as M. stramineus must accomplish this molting phenomenon while clinging to the host. The highest mortality would be expected during each molt.

Table 1 shows that 59 per cent of the started lice were reared to adults in an incubator at 95 per cent relative humidity and 35° C. This was an average for four separate rearings under these conditions. This indicates that 35° C. and 95 per cent relative humidity are near the optimum for M. stramineus when fresh pinfeathers are furnished for food.

Thirty second-generation lice, as well as over 200 first-generation lice, were reared to maturity using the fresh pinfeather as the only source of food. The pinfeather and/or its contents provided the nutrients required for M. stramineus.

repeatedly with the excess ether. The sample was left in the extractor

for six hours and then placed in an oven for three hours to allow the

ether to evaporate. The sample was cooled in a dessicator and then

weighed. The lipid content was calculated by subtracting the post-

extraction weight from the total.

Nitrogen

A micro-Kjeldahl technique was used in the nitrogen determination

(Official Methods of Analysis of the Association of Official Agricultural

Chemists, 1950, 7th ed., pages 745-747). A 10-30 mg sample of the dried

feather tips was weighed on a 3 cm x 3 cm piece of Whatman filter paper,

and the sample plus paper was placed in a Kjeldahl flask. To this flask

1.3 g K_2SO_4, 40 g Cu Selenite, 2 ml H_2SO_4 and two boiling beads were

added. The liquid in the flask was allowed to boil vigorously for one

hour and 15 minutes. After the solution had cooled, 5 ml of water were

added and the flask's contents were transferred to a distillation appara-

tus. A 125 ml Erlenmeyer flask containing 5 ml of 4 per cent boric acid

solution and four drops of indicator was placed under the condensor with

the tip extending below the surface of the liquid. Eight ml of the

$NaOH \cdot Na_2S_2O_3$ reagent were added to the distilation apparatus which was

operated until 14 ml of distillate were collected. The contents of the

receiver flask were diluted to 50 ml and the NH_3 titrated with 0.0222

N. HCl to determine the end point. The per cent protein in the sample was

calculated using the following formula:

$$\% \text{ Nitrogen} = \frac{\text{(ml HCl in detn.) X normality X equivalent wt. Nitrogen X 100}}{\text{Sample wt. (mg)}}$$

$\%$ Protein $= \%$ Nitrogen X Protein Equivalent of Nitrogen

Observation and Discussion

Tables 9, 10 and 11 show in more detail the figures taken during

the analysis of the feather tips for the per cent moisture, lipids and

nitrogen. The one-fourth inch proximal end of the feather yielded an

average of 84.85 per cent water, 10.02 per cent protein and 0.71 per cent

total lipids. The total for these three components is 95.58 per cent.

Ash and carbohydrate content were not analyzed.

Since the pinfeather, (Fig. 6, A, B, C and D) was the only source of

food for rearing over 200 lice from hatching to the adult stage in the

laboratory, it was evident that this food source was essentially furnish-

ing the nutrient requirements. There is always the possibility that

certain trace nutrients or vitamins were not being furnished by the

feather and this deficiency would not appear until later generations.

About 50 second-generation lice were reared to the adult stage from

eggs laid by adults that had been reared in the laboratory. No attempt

was made to rear a third laboratory generation.

Many of the lice that were mounted on slides, including nymphs and

adults, had their intestinal tracts filled with the barbule portion of

feathers (Figs. 13 and 14). Fig. 14 (arrow) shows the teeth of the crop

which hold the undigested feather contents in the crop. Slides made of

the gut contents of the lice were stained with haematoxylin-eosin.[1]

These clearly showed the barbule portion of feathers which were approxi-

mately 325 u long, Fig. 15. This is about one-half the length of the


[1]Stain and staining procedure is given in the Appendix under
Stains.

Fig. 13. Second instar M. stramineus showing the longitudinally arranged feather particles in the crop, 79X

Fig. 14. Crop from second instar M. stramineus, showing the longitudinally arranged feather particles. Arrow shows the crop teeth, which hold the undigested contents in the crop, 400X
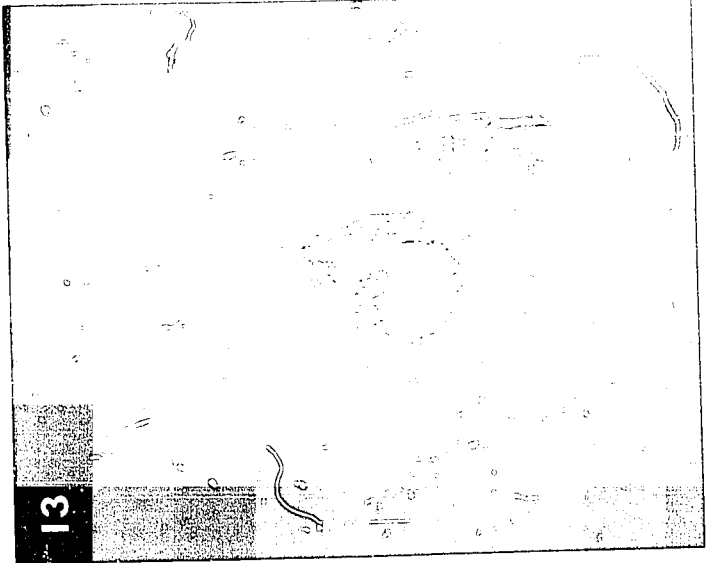
Fig. 15. Crop contents of M. stramineus showing feather barbs, 79X
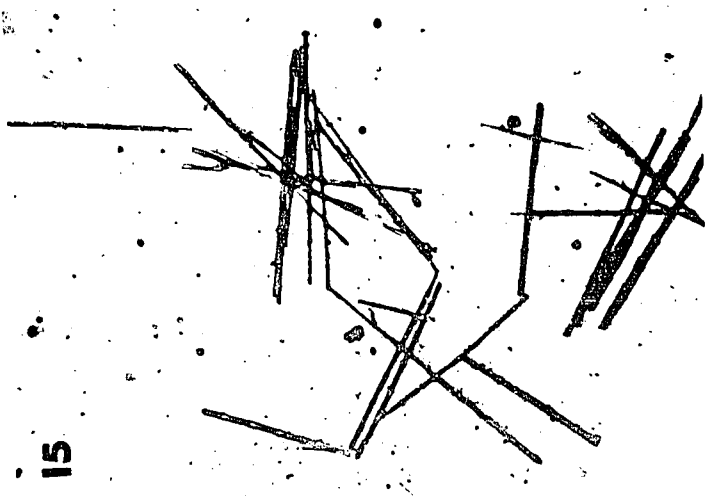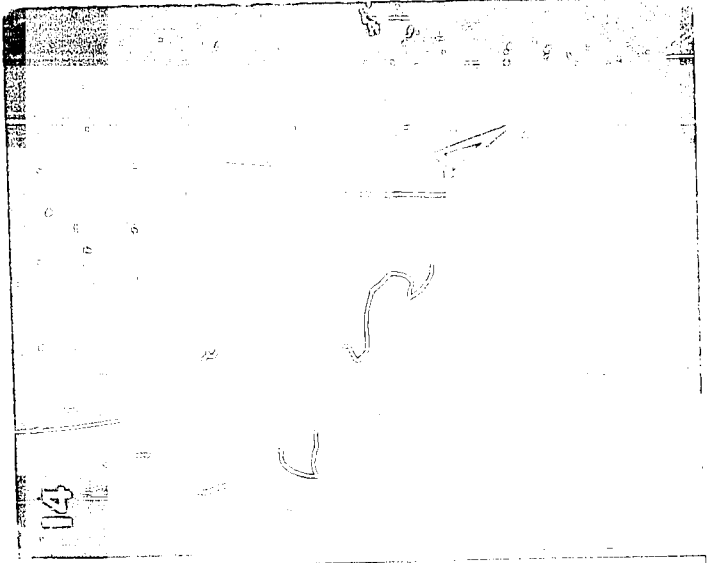
feather barbs reported in the gut of M. stramineus by Waterhouse (1953).

M. stramineus normally eats feather barbules because the intestinal tract is packed with "sticks of feathers". But, since specimens were reared in the laboratory without any barbules or barbs, the shaft and its pulpy contents furnish the necessary nutrients. Most lice in the laboratory were observed feeding at the basal tip of the feather, on either the shaft or the liquid portion. Apparently, the louse is restricted in the chemical composition of its diet but the physical composition of its feed may vary. The liquid constituents are important because no lice were raised past the first instar when fed only the fluffy portion (Fig. 6, F) of the feather.

Sikes (1931) found that the optimum relative humidity for the survival of flea larvae was dependent upon the amount of moisture in the food source. With food of a low moisture content, the larvae required environmental relative humidities of 90 per cent, but when blood albumen was provided, 70 to 80 per cent relative humidity was sufficient.

Buxton (1932) discusses in detail the relationship between the water content of the food of insects and the amount of moisture contained in their environment.

No measurements of the relative humidity of the micro-environment of the louse have been made. One would estimate that the relative humidity of the micro-environment of the louse on the host would not be as high as that relative humidity which has been supplied in the successful in vitro rearing of M. stramineus and other lice.

The moisture in the liquid portion of fresh pinfeathers was high, but would certainly decrease after several hours in an incubator held

at a lower relative humidity. Possibly high relative humidity is necessary to maintain the moisture content of the food source, rather than for actual louse survival in this environment.

Attempts to maintain or rear M. stramineus in vitro were not successful when chicken blood serum or feather meal was the food source even with added moisture. The chicken blood serum was difficult to furnish in small enough quantities to prevent the lice from becoming entangled in it as they attempted to feed. No feeding attempts were noted when dried feather meal was offered as the only source of food.

DISSEMINATION OF LICE

Since many pheasants are reared at game farms and later released in all sections of the state, this could be an important method of dissemination of M. stramineus. A series of tests was designed to see if M. stramineus would establish on the ring neck pheasant. Also, a portion of the wild pheasant population in Iowa was sampled and ecto-parasites removed and examined.

Survey of Adult Pheasants

In an attempt to determine if M. stramineus occurred on the wild population of pheasants in Iowa, 57 birds were washed using a slight modification of the washing technique described on pages 34 and 35. Because of the large size of the adult pheasant, it was placed directly in the specially designed separatory funnel, Fig. 8, and shaken as vigor-ously as possible for approximately five minutes. The ectoparasites were allowed to settle and then drawn off in 50 ml of fluid. The container was labeled and stored for later counting and identification of the lice.

No M. stramineus were found in any of the washings made from 57 birds taken in eight different counties in Iowa during two fall hunting seasons. Four species of lice, commonly found on the pheasant, were re-covered in the washings, however. They were Amyrisidea megalosoma Over-gaard, Lipeurus maculosus Clay, Goniocotes chrysocephalus Giebel, and Goniodes colchici Denny.

Infesting Pheasants with M. stramineus

In Test 8, seven 30 day-old pheasants were obtained from the State Game Farm at Boone, Iowa. These birds were being reared in captivity for later release in the state. The pheasant chicks were poorly feathered apparently due to the overcrowded rearing conditions. The birds were kept in a wire cage isolated from contamination by other lice. Fifteen adult M. stramineus were placed on each chick. The pheasants were so wild that they did not adjust to their new environment, and continually tried to escape by thrusting themselves against the wire-topped pen. Five of the birds died during the 30 days following infestation. As they died, these birds were washed and the washings examined for lice. The remaining two birds were reinfested with 15 adult lice 30 days following the first infestation. Ten days following this second infestation the remaining two birds were killed and washed.

No M. stramineus or pheasant lice were recovered in any of the washings made from the seven pheasants in Test 8 during the test period of one and one-half months.

In Test 9, nine pheasant chicks were obtained from the State Game Farm at Boone, Iowa and nine chickens were obtained from the Ames-In-Cross Hatchery at Roland, Iowa. Both pheasants and chickens were one day old. Ten adult M. stramineus were placed on each of the 18 young birds in this test. At intervals during the ensuing 22 days, one pheasant and one chicken were washed and their lice counted.

M. stramineus became established on the pheasant chicks (Table 5). More lice were recovered from washing the pheasants than the chickens.

Table 5. Lice recovered from pheasants and chickens when washings were made at indicated intervals following artificial infestation

Days following infestation	Louse stages									
	1st		2nd		3rd		Males		Females	
	Ph	Ch	Ph	Ch	Ph	Ch	Ph	Ch	Ph	Ch
1	0	0	0	0	0	0	2	5	1	6
2	0	0	0	0	0	0	0	3	2[a]	3
4	0	0	0	0	0	0	2	0	3	0
8	0	0	0	0	0	0	0	0	2	0
11	10	0	30	3	0	0	0	0	1	0
16	10	0	12	0	17	2	4	0	7	0
18	7	0	11	4	18	6	3	0	9	0
22	8	0	3	0	4	0	6	0	13	1
Total recovered	35	0	56	7	39	8	17	8	38	10

Total recovered from pheasants   185

Total recovered from chickens   33

[a]Two pheasants were washed on this day because no lice were recovered from the first bird washed.

From the nine pheasants 185 lice were recovered, while only 33 were recovered from washing the same number of chickens. The low numbers of adults recovered on the eighth and eleventh days following infestation indicates that the life span on both the host birds may be quite short. This agrees with the observations made in Test 7, page 37.

In Test 10, two adult pheasants were obtained which had been in

captivity for the previous two years. These birds were heavily infested

with _Amyrisidea megalosoma_, a body louse of pheasants. The pheasant lice

were removed from bird A by exposing DDVP[1] vapors on brown wrapping paper

below the wire bottom of the bird's cage for 12 hours. Bird B retained

its lice. The pheasant lice were removed from bird A before infesting

with M. stramineus because it was felt that the adapted _Amyrisidea_

megalosoma lice would prevent establishment of M. stramineus through

competition. Three days following the removal of these pheasant body

lice from bird A, 15 adult M. stramineus were placed on both pheasants.

The birds were left for one month when bird A was sacrificed, washed as

described earlier, and the washings examined for lice. Sixty days fol-

lowing the initial infestation with M. stramineus, bird B's lice were

removed with DDVP vapors and examined. Although the vapor-method of

louse removal was not as thorough in removing all of the lice from a bird,

it did have an advantage in that the bird did not have to be sacrificed.

Also, DDVP vapors did not affect the louse eggs since birds treated in

this manner would soon become repopulated when the louse eggs hatched

although examinations made immediately following treatment would reveal

no living lice.

The only louse species recovered from either bird was _Amyrisidea_

megalosoma.

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[1] 2 2-dichlorovinyl dimethyl phosphate.

Discussion

It is difficult to explain the erratic results which were obtained during this phase of the investigations. The only successful pheasant infestation with M. stramineus was on pheasant chicks which had just hatched (Test 9). The poorly feathered condition of the seven birds in Test 8 could have been the reason for the failure of M. stramineus to infest these birds and the pheasants in Test 10 may have been too old. There may also be a difference in susceptibility of pheasant strains or ages.

Several lice were reared in the laboratory when fed only feathers from a one year old pheasant. This indicates that a mature pheasant can supply the nutritional requirements of M. stramineus.

MORPHOLOGY

Eggs

Eggs laid in the cages were mounted in Hoyers[1] or PVA plus lacto-
phenol. Measurements were made of the width of the eggs at the widest
point. Total length measurements were taken from the posterior tip of
the egg to the anterior portion of the operculum. Several of these eggs
were photographed using Panatomic X film and techniques of photomicro-
graphy.

Measurements for 14 eggs are given in Table 12. The length of the
egg is 0.8725 mm and the width at the widest point is 0.4075 mm. Fig.
16 is a photograph of an egg with the operculum removed. The micropyle
on this operculum shows clearly in the photograph.

The anterior portion of the egg is covered by several rows of
structures which apparently aid in attaching the eggs to feathers and
other eggs. Fig. 17 and Fig. 18 show an egg with several feather barbs
entangled in these "attachment structures". These "attachment fibers"
may also serve another purpose. M. stramineus females lay their eggs
singly and the "attachment structures" are soft and pliable when fresh.
As they dry they radiate outward and harden. Thus when other female lice
attach their eggs next to previously laid eggs, these "attachment struc-
tures" keep the eggs separated and the opercular end from becoming
covered over by later oviposition.

Fig. 19 (arrow) shows some peculiar double-hooked "processes" at

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[1]Formula for the media is given in the Appendix under Mounting Media.

the distal end of the bottom row of "attachment structures". These hooked "processes" become attached to feathers and similar processes from adjacent eggs. Seguy (1951, pages 1341-1364) published drawings of louse eggs and these "hooked processes" are shown on eggs from Menacanthus pallidulus (Neuman) (=Menopon pallidium Piaget) and a Menopon sp. He makes no mention of their function.

At the anopercular end of the egg another structure which apparently aids in the attachment of the egg is shown (Fig. 20). Imms (1960, page 410) refers to this structure as an egg-stigma and describes it "as a group of canals which partly or completely traverse the chorion and which assists in the attachment of the egg".

The operculum structure is shown in Fig. 21. The dome-shaped cap is formed by 8-10 "structures" which are fused together to form the top of the cap and then are only loosely fused, forming a coarse stalk. This cap structure may aid in preventing oviposition in too close proximity for eclosion to occur.

Nymphs

Lice in Test 5 were used for measurements, photographs and drawings. They were placed in 70 per cent alcohol and mounted in PVA plus lactophenol. Measurements of the width of the head, prothorax and metathorax and the length of the head and total length of the body were taken.

Table 12 lists the maximum, minimum and average measurements for the three instars. The width of the head capsule is the best single measure- ment to use to distinguish between instars. The difference between the maximum width of the head capsule of the smaller instar and the minimum

Fig. 16. Egg of M.
           stramineus
           with the
           operculum
           removed.
           Note micro-
           pyle on the
           operculum,
           44X

Fig. 17. Egg of M.
           stramineus
           showing sev-
           eral feather
           barbs entan-
           gled in the
           "structures"
           of the egg,
           53X

Fig. 18. Egg of M.
           stramineus
           showing the
           "attachment
           structures"
           entangled
           with feather
           barbs, 40X

Fig. 19. Egg of M.
           stramineus
           showing the
           peculiar
           "hooked pro-
           cesses",
           116X

Fig. 20. Posterior
           portion of
           the egg of M.
           stramineus
           showing the
           egg stigma,
           567X

Fig. 21. Anterior
           portion of
           M. stramineus
           egg showing
           the structure
           of the oper-
           culum, 70X

16
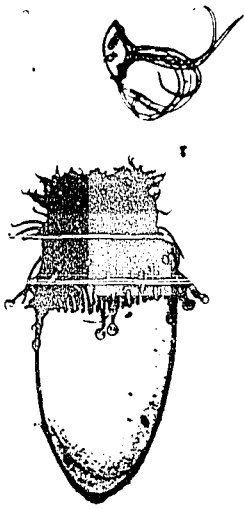
17

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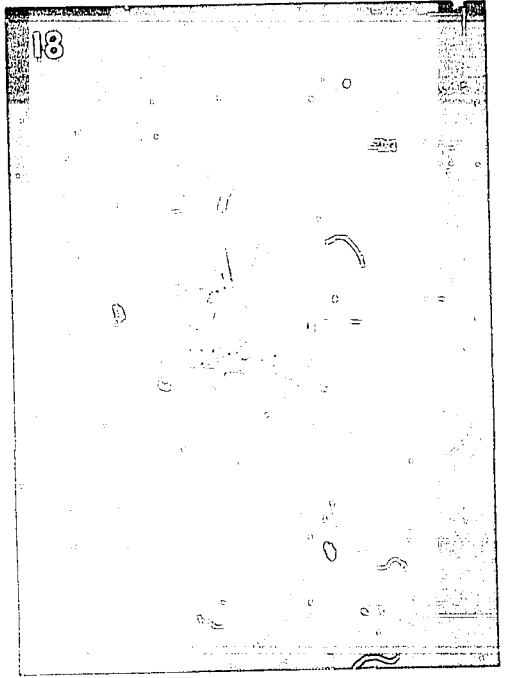
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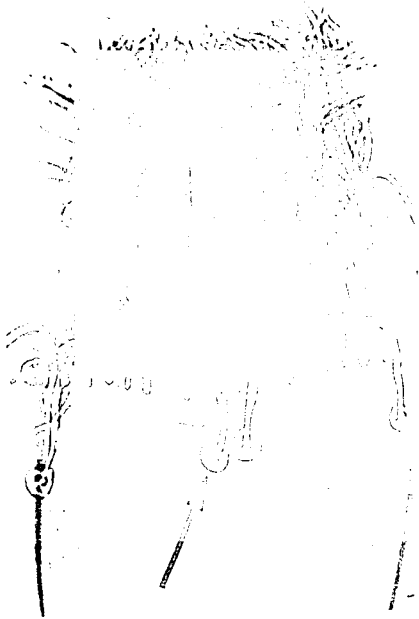
20
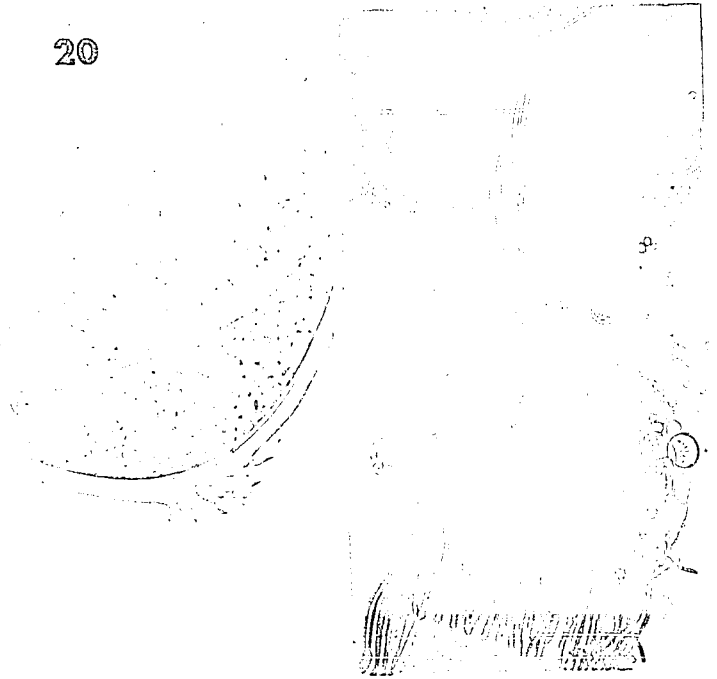
width of the head capsule of the next larger instar is much greater than the corresponding measurements for the prothorax, metathorax, and length of the head and body. The width of the head capsule increases about 0.10 mm with each instar.

Photographs and drawings of the three instars are shown in Figs. 22, 23 and 24. The second instar is larger than the first instar and is slightly darker in color due to the increase in the amount of sclerotization. Both the first and second instar have one row of dorsal setae on each abdominal segment. The chaetotaxy is very similar between these two instars.

The chaetotaxy of the third instar differs considerably from the second instar. It has two dorsal rows of setae on the abdominal segments. The number of setae in each row is approximately double the number of setae in each row of the previous instar. There is also an increase in sclerotization.

Adults

The adults are larger and also have two rows of dorsal setae on each abdominal segment and more sclerotization (Figs. 25 and 26). The male is easily distinguished from the female because of its pointed abdomen. The tip of the female abdomen is rounded. Emerson (1956) and Ansari (1944) have published drawings of the adults. No drawings of the nymphs have previously been published.

Fig. 22a. Photograph of first
instar M. stramineus,
52X

Fig. 22b. Drawing of first
instar M. stramineus,
dorsal view on left,
ventral view on
right, 52X

Fig. 23a. Photograph of second
instar M. stramineus,
38X

Fig. 23b. Drawing of second
instar M. stramineus,
dorsal view on left,
ventral view on
right, 38X

Fig. 24a. Photograph of third
instar M. stramineus,
26X

Fig. 24b. Drawing of third
instar M. stramineus,
dorsal view on left,
ventral view on right,
26X

22a

22b

23a
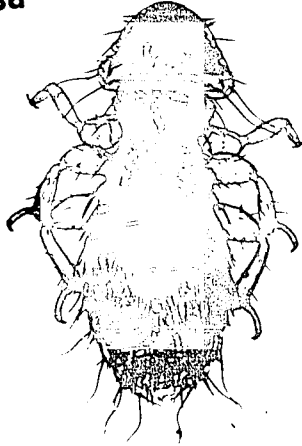
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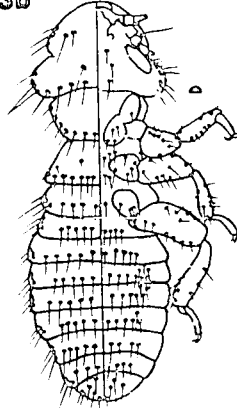
24a
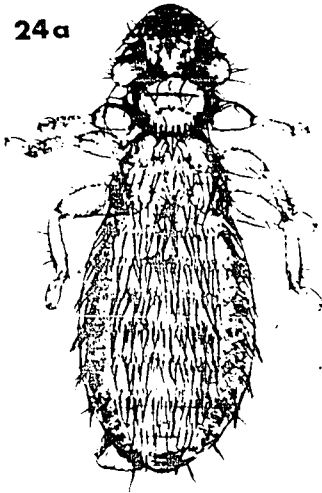
24b
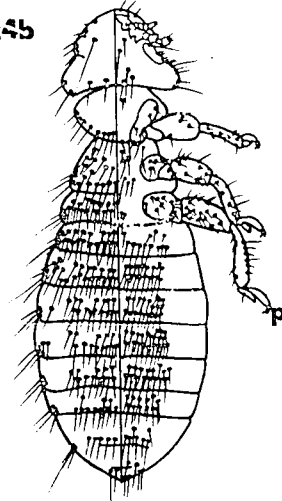
Fig. 25.   Adult <u>M.</u> <u>stramineus</u>
                  female, 35X

Fig. 26.   Adult <u>M.</u> <u>stramineus</u>
                  male, 36X

25
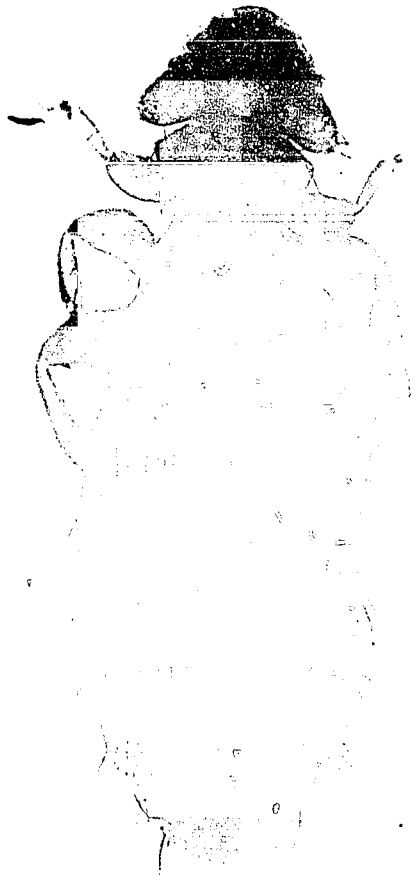
26
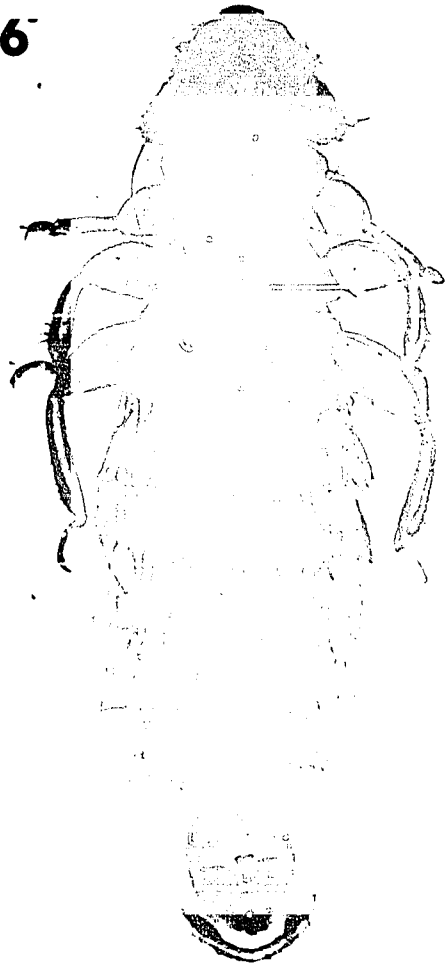
BIOLOGICAL OBSERVATIONS

Rate of Egg Laying and Hatchability

As third instars molted to the adult stage they were removed from their respective rearing cages and mated with a member of the opposite sex. The containers with the paired adult lice were all maintained in the water bath incubator at 35° C. and 95 per cent relative humidity. They were fed once every six hours, or four times a day. In all, 29 pairs of lice were mated. Of these, seven pairs remained alive for at least 10 days and egg laying records were kept.

A few eggs were laid during the first two days of adulthood but only rarely did any of these eggs hatch. These seven pairs of lice laid 136 eggs of which 69 (approximately 50 per cent) hatched. The seven females lived a combined total of 87 days. Thus the egg production per female was 1.57 eggs per day. The rate of oviposition varied from 0 to 4 eggs per day.

In Test 10, in one cage in the water bath at 35° C. and 95 per cent relative humidity, 26 adult lice were reared to maturity. They all molted to the adult stage within a 40-hour period. Once every 24 hours the lice were gently moved into a new container using a camel's hair brush. The number of eggs laid during the 24-hour period was approximated and then incubated under various temperature and humidity conditions. During the period of peak oviposition, it was impossible to accurately count the number of eggs laid, since most viable eggs were glued in masses to the feather. Dismantling the feather to make accurate egg counts would have damaged some of the eggs. The eggs were checked seven times a day during

Table 6. Effects of temperature and humidity on hatchability of M. stramineus eggs

Ages of adults in days	% Rh	Temp. °C.	Days to hatch	Estimated number eggs laid	Number eggs hatching in this batch	Number of viable eggs per female
1	95	35		3	0	
2	95	35		6	0	
3	95	35	5	5	2	.2
4	72	34	5	18	5	.5
5	60	38	4-5	39	36	3.6
6	95	35	4-5	24	41	4.1
7	72	32	6-7	12	17	2.1
8	60	34	6-7	14	11	1.4
9	70	34	5-6	17	15	2.1
10	60	38	5	5	2	.5

the incubation period.

The maximum life span of adult lice in Test 10 was 10 days. This agrees with observations made during in vivo rearing studies (Test 7, Table 4).

The data in Table 6 show that peak oviposition took place when the adults were five and six days old. The data also show that the longest period required for the egg to hatch occurred at temperatures of 32 and $34°$ C. and relative humidities of 72 and 60 per cent respectively.

In several instances, females completely isolated from males oviposited regularly but none of the eggs hatched. Apparently fertilized

eggs are required with this species. Matthysse (1946) found that

Damalinia _bovis_ could reproduce by parthenogenesis. Males were never

found to comprise more than 10 per cent of the population.

Longevity of Reared Adults

On two occasions, males lived 30 days following maturity, or a total

of 43 days in the incubator. This was unusual since most of the lice

died after only 8 to 12 days in the adult stage.

Mating

No reference is made in the literature to indicate that anyone has

observed the mating behavior of _M_. _stramineus_. During the course of these

investigations a portion of coition was observed only once. At the 4 AM

feeding for the 26 adult lice of Test 10, one pair was seen in copulation.

The container holding the lice was gently removed from the water bath,

placed on a slide warmer, and observed under a binocular microscope. The

male was in the more conventional position, dorsal to the female. The tip

of his abdomen was bent ventrally, making contact with the tip of the fe-

male's abdomen, which was arched slightly upward in an accomodating

position. The lice were not moving during this period of observation.

The male's head extended as far forward as the female's thoracic region,

and he gripped the female with his legs.

Since no movement was observed during 10 minutes of observation, a

probe was used to touch the pair. Immediately, they separated and the

aedeagus could clearly be seen as it was removed from the female.

In another observation, a male repeatedly mounted an unreceptive female. The female ran rapidly, dragging the clinging male on her back and eventually rid herself of him. This process was repeated over and over. No penetration of the female was made during the observation.

Molting

The molting process of M. stramineus was observed many times. The skin splits on the dorsum of the head and the split continues posteriorly to the first three to four segments of the abdomen. The head is the first portion to be free of the exuviae which become bent ventrally in the head region and is dragged under the body of the louse by forward movement of the insect. The split in the old skin never reaches the posterior tip of the abdomen. The skin is worked off the last three to four abdominal segments by the forward movement of the louse.

The lice seemed to molt throughout a 24-hour period with no preference for any period of the day.

Oviposition

The length of time required for M. stramineus to lay an egg is undoubtedly very short. No oviposition was seen during many hours of observation in the laboratory.

The eggs were usually fastened to the feather. In the cages, many were glued at the top of the shaft in the lower portion of the feather fluff. From 20 to 40 per cent of the eggs were dropped on the bottom of the cage. Often a few were glued to the basal tip of the feather shaft. The females tended to oviposite at the same site, either on the bolting

silk or some portion of the feather.

Many hours were spent observing M. stramineus eggs but the hatching process was not seen. As the time for hatching nears, the eggs darken in color to a tan or light brown. No clue was observed which would indicate that eclosion was about to take place. This is partially due to the fact that most eggs are observed in a cluster, and only the anterior portion of the egg can be seen because it is surrounded by other hatched and unhatched eggs.

In order to record hatching, a 16 mm movie was taken of this event. Four eggs had been laid over a 12-hour period in an incubator cage. Five days later, two of these eggs had hatched. The third egg hatched while the camera and lights were being oriented. The camera was started to record the events after the operculum of the egg burst open. No air movements in the egg or gut of the louse were noted prior to the bursting open of the operculum. Fig. 27, a, b, c, d, e and f show sequence pictures of some of the frames taken by the movie camera.

Immediately after the operculum was dislodged, a vesicle bulged through the orifice. This vesicle slowly but steadily enlarged, appearing to be blown up like a bladder filled with fluid. Fig. 27, c, (arrow) shows what may be a pre-nymphal skin as the head is being freed from it. The head is folded ventrally onto the thoracic region as reported by Ansari (1944) and Martin (1934).

As soon as the head is extended far enough out of the operculum, it is straightened. The antennae (Fig. 27, d, arrow) and palps move

about readily. The forelegs soon are free from the egg case. The louse moves readily about after freeing itself from the egg case. The whole process from the bursting of the operculum to emergence was completed in less than five minutes.

Eclosion of M. stramineus appeared to be similar to Sikes and Wigglesworth's (1931) description of the hatching process of flea larvae. They were able to observe the larva just prior to its bursting the operculum. The fluid contents of the larva are forced into the head region by antiperistalic movements of the gut. They observed a pre-larval skin, or embryonic cuticle, which still enclosed the larva even after emergence.

Wigglesworth (1932) figures and describes hatching spines on an ichnoceran pigeon louse, Columbicola columbae (=Lipeurus columbae). He describes them as a specialized part of the pre-larval skin that is shed at the time of hatching, and consists of a thickened plate bearing about 18 elongated spines or blades with lance-shaped points. These structures were not observed in M. stramineus.

Fig. 27a, b, c, d, e, and f.   Sequence photographs of the eclosion of M. stramineus, 34X

Arrows indicate: 27c, arrow indicates a membrane which
                        may be an embryonic cuticle

27d, arrow indicates the labial palp

27a 27b 27c 27d 27e 27f
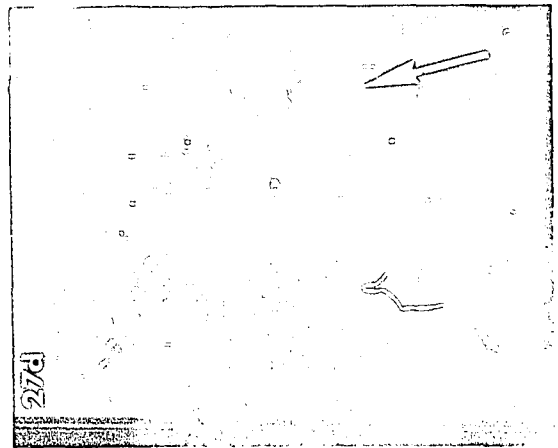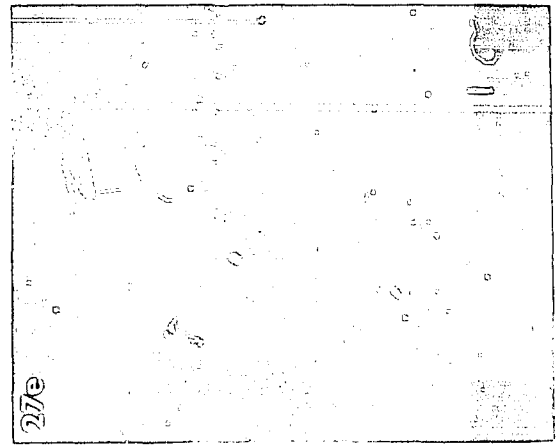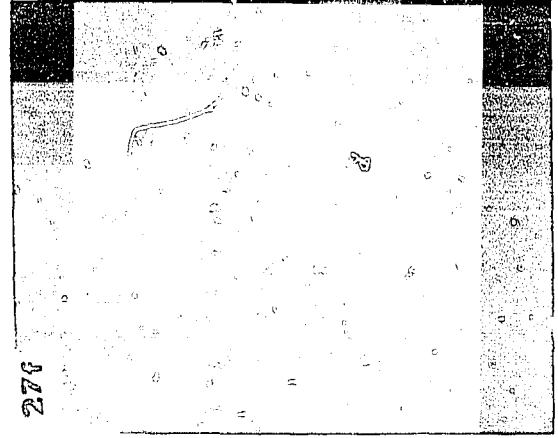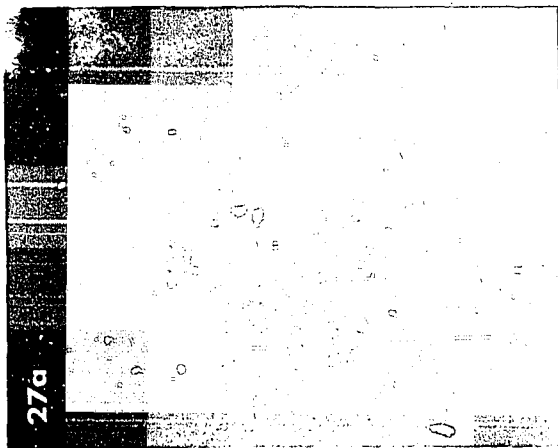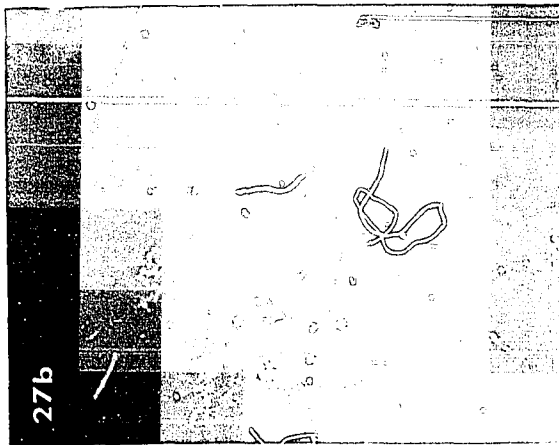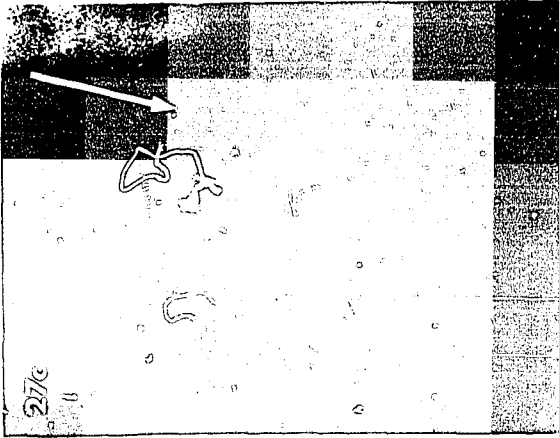
SUMMARY AND CONCLUSIONS

The chicken body louse, Menacanthus stramineus (N.), was studied in vivo and in vitro in these investigations.

M. stramineus appeared best adapted to 35° C. and 95 per cent relative humidity, in laboratory studies. Under these conditions eggs hatched in 5 to 6 days, the three nymphal instars were passed in three days each, and adults lived as long as 30 days. These observations were verified by in vivo studies.

Oviposition records from mated pairs indicated that each female will lay an average of 1.57 eggs per day during her life. Approximately 50 per cent of the eggs hatched in these tests. The highest hatchability occurred from eggs laid by 5- to 6-day old females.

Freshly plucked pinfeathers provided an adequate diet for M. stramineus throughout its life. Since lice could be reared to adulthood on only the basal one-fourth inch of succulent pinfeather, this portion was analyzed for protein (nitrogen), lipid, and water. It contained 84.85 per cent water, 0.71 per cent lipid, and 10.00 per cent protein.

Eclosion was recorded with a 16 mm movie camera. From bursting the operculum to complete emergence from the egg shell, the nymph required less than five minutes to hatch.

Both in vivo and in vitro studies indicate that the ring neck pheasant may at times be a host to M. stramineus and aid in its dissemination to turkey and chicken flocks in Iowa. Examinations of wild pheasants failed to disclose this louse, however.

Morphological observations show that the nymphs of the first two

instars have one row of setae on the dorsal abdominal segments, while

the third instar has the two rows characteristic of adults. The posterior

row of filaments on the eggs were found to have peculiar "attachment

hooks" at the terminal end. These hooks may serve the functions of

attaching eggs to each other and to feathers, and also to prevent eggs

from becoming too closely packed together.

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APPENDIX

Table 7. Life table showing number dying, number surviving, mortality per cent and cumulative mortality of Test 3 for adult M. stramineus fed fresh feathers at 3 hour intervals off the host[a]

Age in days	No. dying			No. surviving			Mortality %			Cumulative mortality		
	ECB slide dryer	Room 109 slide dryer	Inc.	ECB slide dryer	Rm. 109 slide dryer	Inc.	ECB slide dryer	Rm. 109 slide dryer	Inc.	ECB slide dryer	Rm. 109 slide dryer	Inc.
1	5	6	7	27	26	25	25.6	18.8	21.8	5	6	7
2	8	5	7	19	21	18	40.6	34.3	43.7	13	11	14
3	4	6	1	15	15	17	53.1	53.1	46.8	17	17	15
4	1	2	1	14	13	16	56.2	59.3	50.0	18	19	16
5	1	-	1	13	-	15	59.3	-	53.1	19	-	17
6	1	1	1	12	12	14	62.5	62.5	56.3	20	20	18
7	2	1	2	10	11	12	68.7	65.6	62.5	22	21	20
10	0	1	0	10	10	12	68.7	68.7	62.5	22	22	20
13	3	1	5	7	9	7	78.1	71.9	78.1	25	23	25
16	1	3	2	6	6	5	81.3	81.3	84.4	26	26	27
19	0	2	1	6	4	4	81.3	87.5	87.5	26	28	28
22	2	3	1	4	1	3	87.5	96.9	90.6	28	31	29
25	2	1	1	2	0	2	96.9	100.0	96.9	30	32	30
27	0		0	2		2	96.9		96.9	30		30
28		0	0	0		2	100.0		96.9	32		30
30						0			100.0			32

[a]Starting the 10th day, feeding periods were adjusted to 7 during a 24 hour period instead of 8. Feeding periods were midnight, 4, 8 and 11 A.M., 2, 4, and 8 P.M.

Table 8. Life table of adult *M. stramineus* off the host under four different conditions of temperature and humidity

Time in days	No. dying				No. surviving				Mortality percentage				Mortality cumulative			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	2	0	3	0	6	8	5	8	25.0	0	37.5	0	2	0	3	0
2	1	1	1	1	5	7	4	7	37.5	12.5	50.0	12.5	3	1	4	1
4	0	2	2	1	5	5	2	6	37.5	37.5	75.0	25.0	3	3	6	2
6	0	2	0	0	5	3	2	6	37.5	62.5	75.0	25.0	3	5	6	2
8	0	0	0	1	5	3	2	5	37.5	62.5	75.0	37.5	3	5	6	3
10	0	0	0	0	5	3	2	5	37.5	62.5	75.0	37.5	3	5	6	3
12	0	1	0	2	5	2	2	3	37.5	75.0	75.0	62.5	3	6	6	5
14	1	1	0	0	4	1	2	3	50.0	87.5	75.0	62.5	4	7	6	5

A = Slide dryer, 35° C., 66-70 per cent relative humidity

B = Slide dryer, 35° C., 40 per cent relative humidity

C = Incubator, 35° C., 70 per cent relative humidity

D = Water bath, 35°C., 95 per cent relative humidity

Table 9. The average per cent moisture in the basal 1/4-inch of chicken pinfeathers[a]

Sample weight	Sample	
	A	B
Wet weight	0.5907	0.9006
Dry weight	0.0909	0.1348
% moisture	84.6	⌐ 85.1
Average % moisture	84.85	

[a]Measurements given in grams.

Table 10. The average per cent lipids found in the basal 1/4-inch of chicken pinfeathers[a]

	Sample	
	A	B
Sample weight	0.0905	0.1334
Extract weight	0.0034	0.0075
% lipid on a dry weight basis	3.75	5.62
% lipid on a wet weight basis[b]	0.57	0.85
Average % lipid on a wet weight basis	0.71	

[a]Measurements taken in grams.

[b]Calculated by multiplying the per cent lipid on a wet weight basis by 15.15 which was the per cent of the sample which was dry matter.

Table 11. The average per cent nitrogen found in the basal 1/4 inch of chicken pinfeathers[a]

| | Sample | | |
	A	B	C
Sample weight	0.0271	0.0251	0.0270
ml HCl to Titrate	9.6	8.6	8.7
% protein on a dry weight basis	68.85	66.59	62.63
% protein on a wet weight basis[b]	10.43	10.09	9.49
Average % protein on a wet weight basis		10.00	

[a]Measurements taken in milligrams.

[b]Calculated by multiplying the per cent protein on a wet weight basis by 15.15 which was the per cent of the sample which was dry matter.

Table 12. Average measurements in mm of width of head, prothorax, metathorax and length of head and total length of 12 specimens of each instar. Egg length and width measurements were taken on 14 specimens

	First instar	Second instar	Third instar
Width of head			
Maximum	.37	.48	.60
Minimum	.33	.44	.56
Average	.358	.461	.579
Width of prothorax			
Maximum	.28	.39	.50
Minimum	.25	.35	.45
Average	.269	.368	.472
Width of metathorax			
Maximum	.30	.43	.56
Minimum	.27	.39	.50
Average	.293	.419	.535
Length of head			
Maximum	.25	.30	.32
Minimum	.23	.27	.31
Average	.240	.281	.321
Total length			
Maximum	1.17	1.49	2.01
Minimum	.80	1.14	1.47
Average	1.009	1.342	1.777

Egg

Width	
Maximum	.49
Minimum	.38
Average	.407
Length	
Maximum	.92
Minimum	.81
Average	.873

Mounting Media

Hoyer's modification of Berlese's solution

The formula and mixing procedure were adapted from Strandtmann

and Wharton (1958).

Ingredients

Water, distilled. . . . . 50 ml

Gum arabic. . . . . 30 g

Chloral hydrate $(CCl_3 \cdot CH(OH)_2)$. . . . . 200 g

Glycerin $(C_3H_5(OH)_3)$. . . . . 20 g

Mixing procedure   Add gum arabic to distilled water and heat gently

in water bath. Nearly all the gum arabic should be dissolved before the

chloral hydrate and glycerin are added. The resulting solution was

filtered by Schleicher and Schuell analytical filter paper, shark-skin.

Polyvinyl alcohol

Lacto-phenol

Phenol crystals (C_6H_5OH). . . . . 22 g

Lactic acid, 85% $(CH_3 \cdot CH(OH) \cdot COOH)$. . . . . 22 g

PVA

Polyvinyl alcohol, 98% hydrolyzed[1]. . . . . 20 g

Water, distilled, cold. . . . . . . , ... 80 ml

Add the polyvinyl alcohol to the cold distilled water and stir. Heat

the resulting solution in a water bath and stir continuously.

Mixing procedure   Add the entire lacto-phenol solution to 56 g of

the PVA solution and stir.

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[1]Obtained from Matheson Coleman and Bell Division, The Matheson
Company, Inc., East Rutherford, New Jersey.

Stains

Mayer's hematoxylin and eosin

This procedure was adapted from Armed Forces Institute of Pathology (1960, page 27).

Solutions

Mayer's hematoxylin

Hematoxylin (C.I. No. 1246).	1.0 g
Distilled water.	1000.0 ml
Sodium iodate ($NaIO_3$).	0.2 g
Aluminum ammonium sulphate ($AlNH_4(SO_4)_2 \cdot 12H_2O$)	50.0 g
Citric acid ($H_3C_6H_5O_7 \cdot H_2O$)	1.0 g
Chloral hydrate ($CCl_3 \cdot CH(OH)_2$)	50.0 g

Stock 1 per cent alcoholic eosin

Eosin Y (C.I. No. 45380)	1.0 g
Distilled water.	25.0 ml

dissolve, then add:

Ethyl alcohol, absolute.	75.0 ml

Working eosin solution

Stock 1 per cent alcoholic eosin	1 part
Ethyl alcohol, 80 per cent	3 parts

Staining procedure

The slides were stained using the following schedule:

1. Xylene	5 min
2. Ethyl alcohol, absolute.	3 min
3. Ethyl alcohol, 95 per cent	2 min

4. Ethyl alcohol, 70 per cent . . . . 2 min

5. Tap water. . . . . 2 min

6. Mayer's hematoxylin. . . . . 2 min

7. Tap water. . . . . 2 min

8. Working eosin solution . . . . 1 min

9. Ethyl alcohol, 70 per cent . . . . 1 min

10. Ethyl alcohol, 95 per cent . . . . 1 min

11. Ethyl alcohol, absolute. . . . . 1 min

12. Xylene . . . . 2 min

13. Mount in Harleco synthetic resin (HSR)[1], xylene solvent

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[1]Arthur H. Thomas Co., Philadelphia, Pa.